Serum cytokine concentrations, flavonol intake and colorectal adenoma recurrence in the Polyp Prevention Trial

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BACKGROUND: Serum cytokine concentrations may reflect inflammatory processes occurring during the development of colorectal neoplasms. Flavonols, bioactive compounds found in plant-based foods and beverages, may inhibit colorectal neoplasms partly by attenuating inflammation.

METHODS: Using logistic regression, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) to investigate the association between serum concentrations of interleukin (IL)1β, 2, 8, 10, 12p70, granulocyte macrophage colony stimulating factor, interferon-γ, and tumour necrosis factor-α, measured over time, flavonol intake, estimated from a flavonol database used in conjunction with a food frequency questionnaire, and adenoma recurrence in 872 participants from the intervention arm of the Polyp Prevention Trial.

RESULTS: Decreased IL-2 concentration during the trial increased the risk of any adenoma recurrence (4th vs 1st quartile, OR = 1.68, 95% CI = 1.13—2.49), whereas decreased IL-1β or IL-10 reduced the risk of advanced adenoma recurrence (OR = 0.37, 95% CI = 0.15—0.94; OR = 0.39, 95% CI = 0.15—0.98, respectively). Individuals with flavonol intake above the median (29.7 mg per day) and decreased cytokine concentrations had the lowest risk of advanced adenoma recurrence.

CONCLUSION: Overall, no consistent associations were observed between serum cytokine profile and colorectal adenoma recurrence; however, decreased cytokine concentrations during high flavonol consumption may indicate prevention of colorectal neoplasms.

Keywords: cancer prevention; colorectal adenoma; colorectal cancer; cytokines; flavonols

Growing evidence suggests that inflammation is important in carcinogenesis, including colorectal cancer (Lin and Karin, 2007). Cytokine concentrations in either serum or tumours may be useful indicators of inflammation and risk of neoplastic changes (Pellegrini et al, 2006; Cui and Florholmen, 2008). Compared with healthy individuals, serum cytokine concentrations of interleukin-2 (IL-2) are reported to be lower, whereas concentrations of IL-8, IL-10, IL-12, granulocyte macrophage colony stimulating factor (GMCSF), interferon (IFN)-γ, and tumour necrosis factor (TNF)α are higher in individuals with colorectal adenomas in some studies (Berghella et al, 1997; Mroczyk et al, 2001; Galizia et al, 2002; Ordemann et al, 2002; Contasta et al, 2003; Roselli et al, 2003; Kaminska et al, 2005; Kim et al, 2006).

Flavonols are a flavonoid subgroup of bioactive polyphenols that are present in many plant-based foods and beverages (Chun et al, 2007; Bobe et al, 2008). The literature and our own studies suggest that flavonols are one of the flavonoid subgroups most effective in decreasing the risk of advanced and high-risk colorectal adenoma recurrence (Bobe et al, 2008, 2010) and colorectal cancer (Rossi et al, 2006; Theodoratou et al, 2007). Several human studies indicate that flavonols have anti-inflammatory properties (Chun et al, 2008; Boots et al, 2009; Bobe et al, 2010), which may be one of the several molecular mechanisms by which flavonols may inhibit the growth of colorectal neoplasms. The aims of this study were to examine whether serum concentrations of IL-1β, IL-2, IL-8, IL-10, IL-12p70, GMCSF, IFNγ, and TNFα were associated with flavonol intake or could predict colorectal adenoma recurrence. In addition, we investigated whether a predicted protective effect of flavonol intake might be mediated by changes in serum cytokine concentrations.

MATERIALS AND METHODS

Study design and outcome

The Polyp Prevention Trial (PPT) was a 4-year multi-centre, randomised, nutritional intervention trial to evaluate whether colorectal adenoma recurrence can be inhibited by increasing fibre, fruit, and vegetable consumption and decreasing the proportion of fat in the diet. The study has previously been described in detail (Schatzkin et al, 2000; Lanza et al, 2001).
The main requirement was that study participants had at least one histologically confirmed colorectal adenoma identified by complete colonoscopy in the 6 months before study entry. Of the 1905 participants who completed the trial by undergoing a colonoscopy at the end of year 4, 958 were in the intervention arm. Our study included the 872 participants in the intervention arm 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 3 (T3). Two pathologists independently examined all lesions for histological features and degree of atypia. Adenoma recurrence was defined as: any (≥1 adenoma, n = 348), high risk (≥3 adenomas or ≥1 advanced adenoma, n = 100), or advanced (≥1 adenoma of ≥1 cm in size, having ≥25% villous component, or exhibiting high-grade dysplasia, n = 49). The institutional review boards of the National Cancer Institute and each participating centre approved the study, and all participants provided written informed consent.

Lifestyle and flavonol data

At T0 and at each of the annual follow-up visits (T1, T2, T3, and T4), participants were asked to complete an interviewer-administered questionnaire about demographics, family history, and use of medication or supplements (including name and dosage), as well as a self-administered food frequency questionnaire (FFQ) that was reviewed with a certified nutritionist. The FFQ was specifically designed and validated to accurately measure fat, fibre, fruit, and vegetable consumption (Block et al., 1990). Relative to 24-h dietary recall and 4-day food record data, the FFQ slightly overestimated fat and underestimated fibre, fruit and vegetable intake, and had acceptable correlations of macronutrients and micronutrients (Caan et al., 1999; Lanza et al., 2001). The average flavonol intake for the first 3 years of the trial was estimated using 55 of the 119 questions on the FFQ using the 2007 flavonoid database (U.S. Department of Agriculture, 2007) and was calculated as the sum of isorhamnetin, kaempferol, myricetin, and quercetin.

Serum data

At each annual visit, participants provided an overnight fasting blood sample, the serum from which was stored at −70°C until analysis. Among the 872 participants, 23 and 69 had no available samples at T1 and T3, respectively. Serum concentrations of IL-1β, IL-2, IL-8, IL-10, IL-12p70, GMCSF, IFNγ, and TNFα were measured at T0, T1, and T3 by the Clinical Support Laboratory of SAIC Frederick, Inc. (Frederick, MD, USA) using a commercially available multiplex 96-well enzyme-linked immunosorbent assay kit (MS0600 Human Pro-Inflammatory 9-Plex Ultra-Sensitive Kit K111007; Meso Scale Diagnostics, Gaithersburg, MD, USA) on a Sector Imager 6000 according to the manufacturer’s recommendation (Meso Scale Diagnostics). Study samples were run with two pooled serum samples and three assay specific standards in duplicate and the average of the duplicate was used. Fewer than 1% of the samples were below the detection limit, and the interassay coefficient of variation (CV) was below 15%.

Statistical analyses

Statistical analyses were performed using SAS, version 9.1 (SAS, Inc., Cary, NC, USA) software. Baseline characteristics, average dietary intake for the first 3 years of the trial, and serum cytokine concentrations were evaluated by adenoma recurrence at T4 (no vs any, high-risk, or advanced adenoma recurrence) using Wilcoxon rank-sum test for continuous variables and Fisher’s exact test for categorical variables and are shown as medians and interquartile ranges (IQRs). Spearman’s correlation coefficients between serum cytokine concentrations were calculated. The association between serum cytokine concentrations and flavonol consumption during the first 3 years of the trial was evaluated with the Kruskal–Wallis test and multiple linear regression models.

We defined trial cytokine concentrations as the geometric mean of T1 and T3. Cytokine concentration changes during the trial were defined as the geometric mean of T1 and T3 minus the baseline values. The association between cytokine changes and colorectal adenoma recurrence was estimated by odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression. A trend test was performed using the median values of each quartile as a continuous variable in a logistic regression model. The median values of both flavonol intake and cytokine changes were used as cutoffs (≤ median, > median) to examine the combined effect of flavonol intake and cytokine changes on colorectal adenoma recurrence. Potential confounders (listed in Table 1) were added to the models in a stepwise manner and remained in the model if they changed the association by >10%, were associated with both study variables, and had a χ² P-value ≤ 0.20. All P-values corresponded to two-sided tests and were considered to be significant when P ≤ 0.05.

RESULTS

At the end of the 4-year trial, 40% of participants had at least 1 adenoma, 11% had high-risk adenoma, and 6% had an advanced adenoma recurrence (Table 1). Compared with baseline, flavonol consumption increased two-fold from 14.6 to 29.7 mg per day during the first 3 years of the trial (Bobe et al., 2010). Adenoma recurrence was more common in men, older individuals, and individuals that ate a greater percentage of calories from fat during the first 3 years of the trial, and less common in women who used hormone therapy. Individuals who had recurrence of a high-risk or advanced adenoma consumed less fibre (limited to individuals with a high-risk adenoma), fruits and vegetables, flavonols, and dry beans (Table 1). Serum concentrations of IL-1β, IL-2, IL-8, IL-10, GMCSF, IFNγ, and TNFα, either at baseline, during the first 3 years of the trial, or from baseline to during the trial, were not associated with colorectal adenoma recurrence; with the exception that IL-12p70 was lower at baseline in individuals with high-risk and advanced adenoma recurrence than in individuals with no adenoma recurrence (Table 1; data not shown). Of the eight serum cytokines measured, only IFNγ concentrations differed across quartiles of flavonol intake; individuals in the lowest flavonol intake quartile had higher IFNγ concentrations compared with individuals in the higher 3 flavonol intake quartiles (Table 2).

No statistically significant associations were observed between serum cytokine concentrations during the trial (defined as the mean of concentration at T1 and T3) and adenoma recurrence (data not shown). In contrast, a decrease in IL-2 concentrations during the trial (the mean trial level minus the baseline concentration) was associated with increased risk of any adenoma recurrence (lowest vs highest quartile of change in cytokine concentration: OR = 1.68, 95% CI = 1.13–2.49), whereas a decrease in IL-1β or IL-10 reduced the risk of advanced adenoma recurrence (OR = 0.48, 95% CI = 0.25–0.95) (Figure 1; Supplementary Table S1). Individual with above median flavonol intake and equal or below median change in serum cytokine concentrations had the lowest risk of advanced adenoma recurrence for all cytokines investigated but not all were statistically significant (Figure 1). Compared with individuals with equal or below median flavonol intake and above median serum cytokine concentrations, the risk reduction was statistically significant for changes in concentrations of IL-1β, IL-10, IL-12p70, GMCSF, IFNγ, or TNFα (Figure 1; Supplementary Table S1). Similar results were observed for the combined effect of flavonol intake and serum cytokine concentrations at baseline (less significant effect) or during the trial (Supplementary Tables S2 and S3).
Previously, we reported that serum concentrations of IL-6 may be associated with colorectal adenoma recurrence with the exception that a decrease in IL-2 concentrations during the trial increased the risk of any adenoma recurrence, and a decrease in IL-1 \( \beta \) concentrations varied significantly across flavonol intake quartiles. Serum cytokine concentrations were not associated with colorectal adenoma recurrence with the exception that a decrease in IL-2 concentrations during the trial increased the risk of any adenoma recurrence, and a decrease in IL-1/\(-\beta\) or IL-10 reduced the risk of advanced adenoma recurrence. Individuals with high flavonol intake (above 29.7 mg per day) and a decrease in serum concentrations of six of the eight measured cytokines had the lowest risk of advanced adenoma recurrence. Thus, our results suggest that there is not a consistent association between serum cytokine profile and colorectal adenoma recurrence; however,

\[\text{DISCUSSION}\]

Previously, we reported that serum concentrations of IL-6 may be a potential risk indicator for advanced and high-risk adenoma recurrence; furthermore, dietary flavonols decrease elevated IL-6 concentrations and decrease the risk of advanced and high-risk adenoma recurrence (Bobe et al., 2010). In the current study, we examined serum concentrations of eight cytokines (IL-1/\(-\beta\), IL-2, IL-8, IL-10, IL-12p70, GMCSF, IFN\(\gamma\), and TNF\(\alpha\)) in relation to flavonol intake and colorectal adenoma recurrence and found none to be associated with flavonol intake and with colorectal adenoma recurrence. Only IFN\(\gamma\) concentrations varied significantly across flavonol intake quartiles. Serum cytokine concentrations were not associated with colorectal adenoma recurrence with the exception that a decrease in IL-2 concentrations during the trial increased the risk of any adenoma recurrence, and a decrease in IL-1/\(-\beta\) or IL-10 reduced the risk of advanced adenoma recurrence. Individuals with high flavonol intake (above 29.7 mg per day) and a decrease in serum concentrations of six of the eight measured cytokines had the lowest risk of advanced adenoma recurrence. Thus, our results suggest that there is not a consistent association between serum cytokine profile and colorectal adenoma recurrence; however,
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potential of IL-8, TNF

Schetter

Contasta

(2007).

lower serum concentrations of IL-2

to promote colorectal carcinogenesis (Lin and Karin,

Similar to our findings, lower serum concentrations of IL-2

expression of IL-1β

carcinoma relative to normal colon tissue (Miki

Although we did not find associations for IL-8, IL-12p70,

TNFα, and TNFβ and adenoma recurrence, they may serve

interleukin-1β

Interleukin-2

Interleukin-8

Interleukin-10

Interleukin-12p70

Interferon-γ

Tumour necrosis factor α

IL-2 is a lymphokine that enhances the growth and cytotoxic

cascade and is necessary for tumour invasion and metastasis (Apte

Chronic inflammation, involving many pro- as well as anti-

inflammatory cytokines, is one of the many mechanisms reported

expression risk, or flavonol-induced changes in inflammatory markers

attenuation of cytokine secretion and gene expression. Thus,

a decrease in cytokine concentrations during high flavonol

consumption (> 29.7 mg per day) may indicate a lower risk for

advanced colorectal adenoma.

Participants were grouped in quartiles (Q1 – Q4) by mean flavonol intake during the first 3 trial years. c P-values for differences in medians among the flavonol intake quartiles were calculated based on the Kruskal-Wallis test. d Median concentrations of each flavonol quartile were used to determine P for trend of the cytokine concentrations using a multiple regression model adjusting for age tertiles (< 58, 58–66, > 66 years), sex, average BMI (< 25, 25.0–29.9, ≥ 30 kg m–2), smoking status, and average energy intake (continuous) during the first 3 trial years. Individuals in the lowest flavonol intake quartile had higher interferon-γ concentrations than individuals in the three higher flavonol intake quartiles, while not differing among each other.

\[ P_{\text{non-param}} = 0.0003, \quad P_{\text{for trend}} = 0.03 \]

Table 2

| Cytokine* (pg ml–1) | Q1: < 21.1 Median (IQR) | Q2: 21.1–29.6 Median (IQR) | Q3: 29.7–40.0 Median (IQR) | Q4: > 40.0 Median (IQR) | P non-param. | P for trend |
|---------------------|-------------------------|-----------------------------|-----------------------------|-------------------------|-------------|------------|
|                     | 218                     | 218                         | 218                         | 218                     |             |            |
| Interleukin-1β      | 0.41 (0.26–0.70)        | 0.36 (0.23–0.61)            | 0.35 (0.23–0.51)            | 0.35 (0.22–0.62)        | 0.14        | 0.09       |
| Interleukin-2        | 0.80 (0.38–1.65)        | 0.74 (0.30–1.41)            | 0.78 (0.39–1.26)            | 0.77 (0.37–1.66)        | 0.69        | 0.28       |
| Interleukin-8        | 10.8 (8.42–14.7)        | 11.0 (8.72–16.0)            | 10.5 (7.85–15.0)            | 10.5 (7.95–15.2)        | 0.62        | 0.90       |
| Interleukin-10       | 3.41 (2.26–6.11)        | 3.02 (2.07–4.96)            | 3.15 (2.15–6.40)            | 3.27 (2.19–5.58)        | 0.53        | 0.81       |
| Interleukin-12p70    | 3.17 (1.70–7.85)        | 2.73 (1.48–5.53)            | 3.06 (1.70–7.25)            | 3.02 (1.48–7.84)        | 0.15        | 0.92       |
| GMCSF                | 0.82 (0.43–1.78)        | 0.70 (0.38–1.48)            | 0.82 (0.38–2.34)            | 0.83 (0.41–2.05)        | 0.35        | 0.74       |
| Interferon-γ         | 1.61 (1.31–2.64)        | 1.20 (0.78–2.06)            | 1.37 (0.91–2.15)            | 1.28 (0.89–1.98)        | 0.0003      | 0.03       |
| Tumour necrosis factor α | 8.06 (6.68–10.2)     | 8.52 (7.05–10.2)            | 8.02 (6.93–9.71)            | 8.15 (6.95–9.65)        | 0.46        | 0.90       |

Abbreviations: GMCSF = granulocyte macrophage colony stimulating factor; IQR = interquartile range. *Geometric mean of years 1 and 3 cytokine values (Trial 1,3). ** Participants were grouped in quartiles (Q1 – Q4) by mean flavonol intake during the first 3 trial years. P-values for differences in medians among the flavonol intake quartiles were calculated based on the Kruskal-Wallis test. Median concentrations of each flavonol quartile were used to determine P for trend of the cytokine concentrations using a multiple regression model adjusting for age tertiles (< 58, 58–66, > 66 years), sex, average BMI (< 25, 25.0–29.9, ≥ 30 kg m–2), smoking status, and average energy intake (continuous) during the first 3 trial years. Individuals in the lowest flavonol intake quartile had higher interferon-γ concentrations than individuals in the three higher flavonol intake quartiles, while not differing among each other.
anti-inflammatory properties, dietary flavonols are thought to inhibit carcinogenesis through several other pathways. Flavonols can decrease various forms of DNA damage (Duthie and Dobson, 2009), inhibit carcinogenesis through several other pathways. Flavonols can scavenge reactive oxygen species (Kim et al, 2006; Wang et al, 2010), stabilise the helical structure of DNA (et al, 2007), and enhance DNA repair (Min and Ebeler, 2007), and repress expression of the angiogenesis-inducing cell cycle arrest and apoptosis (Choi 2007; Lee et al, 2008; Jeong et al, 2009). In the tumour promotion and progression stage, flavonols inhibit transformation of pre-carcinogenic cells (Ichimatsu et al, 2007; Lee et al, 2008) and proliferation of cancer cells (Richter et al, 1999; Kim et al, 2005) by inducing cell cycle arrest and apoptosis (Choi et al, 2008; Jeong et al, 2009).

Furthermore, flavonols can scavenge reactive oxygen species (Kim et al, 2006; Wang et al, 2006), bind metals (Guo et al, 2007), decrease lipid peroxidation (Lee et al, 2010c), inhibit the activity of phase I procarcinogen activating enzymes (Si et al, 2009; Lam et al, 2010; Tiong et al, 2010), and induce the expression of phase II carcinogen detoxification enzymes (Lam et al, 2010) and antioxidant proteins (Kimura et al, 2009). In the tumour promotion and progression stage, flavonols inhibit transformation of pre-carcinogenic cells (Ichimatsu et al, 2007; Lee et al, 2008) and proliferation of cancer cells (Richter et al, 1999; Kim et al, 2005) by inducing cell cycle arrest and apoptosis (Choi et al, 2008; Jeong et al, 2009).

### Table 3

Association between quartiles of change in serum cytokine concentrations from baseline to the levels measured during the trial (mean of T1 and T3) and colorectal adenoma recurrence in the intervention arm of the Polyp Prevention Trial (n = 872).

| Cytokine (pg ml⁻¹) | None (n) | Any (n) | High risk (n) | Advanced (n) |
|-------------------|----------|---------|--------------|--------------|
| **Interleukin-1β** |
| Q1: >0.16         | 136 (62.7) | 81 (37.3) | 29 (13.4) | 18 (8.3) |
| Q2: 0.02–0.16     | 133 (61.0) | 85 (39.0) | 22 (10.1) | 9 (4.1) |
| Q3: –0.13–0.01    | 128 (58.7) | 90 (41.3) | 31 (14.2) | 15 (6.9) |
| Q4: <–0.13        | 127 (58.0) | 92 (42.0) | 18 (8.2) | 7 (3.2) |
| P for trenda      | 0.40      | 0.18     | 0.06         | 0.06         |
| **Interleukin-2** |
| Q1: >0.41         | 142 (65.4) | 75 (34.6) | 25 (11.5) | 13 (6.0) |
| Q2: 0.07–0.41     | 136 (61.3) | 86 (38.4) | 21 (9.6) | 9 (4.1) |
| Q3: –0.30–0.06    | 133 (61.0) | 87 (39.0) | 30 (13.8) | 15 (6.9) |
| Q4: <–0.30        | 117 (53.4) | 102 (46.6) | 23 (10.5) | 14 (6.4) |
| P for trenda      | 0.01      | 0.31     | 0.52         | 0.04         |
| **Interleukin-8** |
| Q1: >2.74         | 137 (63.1) | 80 (36.9) | 21 (9.7) | 8 (3.7) |
| Q2: 0.25–2.74     | 128 (58.7) | 90 (41.3) | 20 (9.2) | 8 (3.7) |
| Q3: –2.02–0.24    | 121 (55.9) | 94 (43.1) | 30 (13.8) | 12 (5.5) |
| Q4: <–2.02        | 135 (61.6) | 84 (38.4) | 29 (13.2) | 14 (6.4) |
| P for trenda      | 0.83      | 0.27     | 0.24         | 0.04         |
| **Interleukin-10**|
| Q1: >0.57         | 135 (62.2) | 82 (37.8) | 27 (12.4) | 18 (8.3) |
| Q2: –0.03–0.57    | 127 (58.3) | 91 (41.7) | 26 (11.9) | 12 (5.5) |
| Q3: –0.88 to –0.04| 133 (61.0) | 85 (39.0) | 24 (11.0) | 12 (5.5) |
| Q4: < –0.88       | 129 (59.8) | 90 (41.1) | 23 (10.5) | 9 (4.1) |
| P for trenda      | 0.50      | 0.62     | 0.04         | 0.06         |
| **Granulocyte macrophage colony stimulating factor** |
| Q1: >0.68         | 137 (63.1) | 80 (36.9) | 24 (11.1) | 15 (6.9) |
| Q2: –0.08–0.68    | 127 (58.5) | 92 (42.2) | 32 (14.7) | 16 (7.3) |
| Q3: –1.03 to –0.09| 128 (58.7) | 90 (41.3) | 21 (9.1) | 12 (5.5) |
| Q4: <–1.03        | 133 (60.7) | 86 (39.3) | 20 (9.1) | 12 (5.5) |
| P for trenda      | 0.78      | 0.43     | 0.21         | 0.06         |
| **Interferon-γ**  |
| Q1: >0.57         | 134 (61.5) | 84 (38.5) | 26 (11.9) | 16 (7.3) |
| Q2: 0.08–0.57     | 131 (60.4) | 87 (39.6) | 27 (12.4) | 13 (6.0) |
| Q3: –0.39–0.07    | 122 (60.4) | 85 (39.4) | 25 (11.5) | 15 (6.9) |
| Q4: <–0.39        | 127 (58.0) | 92 (42.0) | 22 (10.0) | 12 (5.5) |
| P for trenda      | 0.49      | 0.61     | 0.18         | 0.36         |
| **Tumour necrosis factor α** |
| Q1: >0.87         | 129 (59.4) | 88 (40.6) | 21 (9.7) | 12 (5.5) |
| Q2: 0.03–0.87     | 131 (60.1) | 87 (39.9) | 29 (13.3) | 15 (6.9) |
| Q3: –0.90–0.02    | 128 (58.7) | 90 (41.3) | 27 (12.4) | 15 (6.9) |
| Q4: <–0.90        | 136 (62.1) | 83 (37.9) | 23 (10.5) | 10 (4.6) |
| P for trenda      | 0.40      | 0.76     | 0.56         | 0.56         |

Abbreviations: CI = confidence interval; OR = odds ratio. *Change in cytokine values is defined as difference between the geometric mean value of years 1 and 3 and baseline. **Multivariate OR and 95% CI models were adjusted for age tertiles (<58, 58–66, >66 years), sex, average BMI (<23, 25.0–29.9, >30 kg m⁻²), and current smoking status during the first 3 trial years. *Median concentrations of each quartile were used to determine P for trend for the change in cytokine concentrations.
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Figure 1  Association between the combination of high (> 29.7 mg per day) or low (≤ 29.7 mg per day) flavonol intake during the trial and change in serum concentration of cytokines (defined as the geometric mean of T1 and T3 minus baseline values) on advanced colorectal adenoma recurrence among participants in the intervention arm of the Polyp Prevention Trial. The cutoff values for an increase or decrease in serum cytokine concentrations are as follows (in pg ml⁻¹): ΔIL-1β: > 0.01 (increase), ≤ 0.01 (decrease); ΔIL-2: > 0.06 (increase), ≤ 0.06 (decrease); ΔIL-8: > 0.24 (increase), ≤ 0.24 (decrease); ΔIL-10: > −0.04 (increase), ≤ −0.04 (decrease); ΔIL-12p70: > −0.09 (increase), ≤ −0.09 (decrease); ΔGMCSF: > 0.00 (increase), ≤ 0.00 (decrease); ΔIFNγ: > 0.07 (increase), ≤ 0.07 (decrease); TNFα: > 0.02 (increase), ≤ 0.02 (decrease). The reference group is the combination of low flavonol intake and increase in cytokine concentrations.

2008; Phromnoi et al, 2009; Zhang and Zhang, 2009). Thus, the decrease in cytokine concentrations may be, at least in part, a result of flavonols inhibiting adenoma progression rather than a direct effect on cytokine expression and secretion.

One of the strengths of this study is the detailed end point information, which included complete colonoscopies and histologic characterisation of all lesions by two pathologists, decreasing the risk of misclassification. A second strength is the prospective and repeated collection of dietary exposure. The modified FFQ used in the PPT was specifically developed to accurately measure high fruit and vegetable consumption (Block et al, 1990; Lanza et al, 2001) and was linked to the recently released validated USDA flavonoid database (U.S. Department of Agriculture, 2007). The accuracy of the FFQ was further improved as registered dieticians reviewed the FFQ with participants (Caan et al, 1999). A third strength is the repeated collection of serum, which allowed us to look at changes during, what may be, early stages of colorectal carcinogenesis.

Limitations of the study include the fact that the PPT is a study of individuals with a history of adenomas, most of whom were...
Caucasians already engaged in a health-promoting lifestyle. Random as well as systematic measurement error related to the dietary assessment, the flavonoid database, and the participants’ knowledge of the expected dietary patterns may be present and could bias risk estimates. The low abundance, high CVs, daily fluctuations, short half-lives, lack of specificity for location, strength and type of inflammation, and the limited dynamic ranges of cytokines in most human serum samples could partly explain the inconsistent results for serum cytokines as markers of colorectal neoplasia and limit the usefulness of many cytokines as biomarkers. Observed differences may have arisen by chance as participants were not randomly assigned to a specific flavonol diet, the number of cases of advanced adenoma recurrence was small, and multiple cytokines were tested for multiple outcomes (multiple testing). However, the consistent lower risk of advanced adenoma recurrence with decreasing cytokine concentration during high flavonol consumption is unlikely due to chance. Besides flavonols, other flavonoid subgroups, such as anthocyanins, flavan-3-ols, flavones, and isoflavonoids, have cancer-protective and anti-inflammatory properties (Yoon and Baek, 2005; Ferguson and Philpott, 2007; Wang and Stoner, 2008). We focused on flavonols because they were the flavonoid subgroup most protective against advanced adenoma recurrence in the PPT (Bobe et al., 2008); the intake ranges of other flavonoid subgroups in the PPT may be too limited to detect associations.

REFERENCES

Apte RN, Dotan S, Elkahets M, White MR, Reich E, Carmi Y, Song X, Dvozkin T, Krelin Y, Voronov E (2006) The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer Metastasis Rev 25: 387 – 408

Bandyopadhyay S, Romero JR, Chattopadhyay N (2008) Quercetin stimulates granulocyte-macrophage colony-stimulating factor secretion in human prostate cancer cells. Mol Cell Endocrinol 287: 57 – 64

Bednar W, Holzmann K, Marian B (2007) Assessing 12(S)-lipoxigenase inhibitory activity using colorectal cancer cells overexpressing the enzyme. Food Chem Toxicol 45: 508 – 514

Berghella AM, Contasta I, Pellegrini P, Del Beato T, Adorno D (2002) Peripheral blood immunological parameters for use as markers of pre-invasive to invasive colorectal cancer. Cancer Biother Radiopharm 17: 43 – 50

Berghella AM, Pellegrini P, Del Beato T, Adorno D, Casciani CU (1997) IL-10 and sIL-2R serum levels as possible peripheral blood prognostic markers in the passage from adenoma to colorectal cancer. Cancer Biother Radiopharm 12: 265 – 272

Berghella AM, Pellegrini P, Del Beato T, Maccarone D, Adorno D, Casciani CU (1996) Prognostic significance of immunological evaluation in colorectal cancer. Cancer Biother Radiopharm 11: 355 – 361

Block G, Hartman AM, Naughton D (1990) A reduced dietary questionnaire: development and validation. Epidemiology 1: 58 – 64

Boe G, Albert PS, Sansbury LB, Lanza E, Schatzkin A, Colburn NH, Cross AJ (2010) Interleukin-6 as a potential indicator for prevention of high-risk adenoma recurrence by dietary flavonols in the polyp prevention trial. Cancer Prev Res (Phila Pa) 3: 764 – 775

Boe G, Sansbury LB, Albert PS, Cross AJ, Kahle L, Ashby J, Slattery ML, Caan B, Paskett E, Iber F, Kikendall JW, Lance P, Daston C, Marshall JR, Schatzkin A, Lanza E (2008) Dietary flavonoids and colorectal adenoma recurrence in the Polyp Prevention Trial. Cancer Epidemiol Biomarkers Prev 17: 1344 – 1353

Boots AW, Drent M, Swennen EL, Moonen HJ, Bast A, Haenen GR (2009) Antioxidant status associated with inflammation in sarcoidosis: a potential role for antioxidants. Respir Med 103: 364 – 372

Boots AW, Wilms LC, Swennen EL, Kleinjans JC, Bast A, Haenen GR (2008) In vitro and ex vivo anti-inflammatory activity of quercetin in healthy volunteers. Nutrition 24: 703 – 710

Caan BJ, Lanza E, Schatzkin A, Coates AO, Brewer BK, Slattery ML, Marshall JR, Bloch A (1999) Does nutritionist review of a self-administered food frequency questionnaire improve data quality? Public Health Nutr 2: 565 – 569

Camuemos D, Comalada M, Concha A, Nieto A, Sierra S, Xaus J, Zarruzelo A, Galvez J (2006) Intestinal anti-inflammatory activity of combined quercetin and dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polysaturated fatty acids, in rats with DSS-induced colitis. Clin Nutr 25: 466 – 476

Choi EJ, Bae SM, Ahn WS (2008) Antiproliferative effects of quercetin through cell cycle arrest and apoptosis in human breast cancer MDA-MB-435 cells. Arch Pharm Res 31: 1281 – 1285

Chun OK, Chung SJ, Claycombe KJ, Song WO (2008) Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. J Nutr 138: 753 – 760

Chun OK, Chung SJ, Song WO (2007) Estimated dietary flavonoid intake and major food sources of U.S. adults. J Nutr 137: 1244 – 1252

Contasta I, Berghella AM, Pellegrini P, Adorno D (2003) Passage from normal mucosa to adenoma and colon cancer: alteration of normal sCD30 mechanisms regulating TH1/TH2 cell functions. Cancer Biother Radiopharm 18: 549 – 557

Csiszar A, Szentes T, Haraszti B, Balazs A, Petryani GG, Pocsik E (2004) The pattern of cytokine gene expression in human colorectal carcinoma. Pathol Oncol Res 10: 109 – 116

Cui G, Florholmen J (2008) Polarization of cytokine profile from Th1 into Th2 along colorectal adenoma-carcinoma sequence: implications for the biotherapeutic target? Inflamm Allergy Drug Targets 7: 94 – 97

Duthie SJ, Dobson VL (1999) Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr 38: 28 – 34

Egert S, Boesch-Saadatmandi C, Wolffram S, Rimbach G, Muller MJ (2010) Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. J Nutr 140: 278 – 284

Ferguson LR, Philpott M (2007) Cancer prevention by dietary bioactive components that target the immune response. Curr Cancer Drug Targets 7: 459 – 464

Galizia G, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, De Vita F (2002) Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. Clin Immunol 102: 169 – 178

Grande C, Firvida JL, Navas V, Casal J (2006) Interleukin-2 for the treatment of solid tumors other than melanoma and renal cell carcinoma. Anticancer Drugs 17: 1 – 12

Guo M, Perez C, Wei Y, Rapoza E, Su G, Bou-Abdallah F, Chasteen ND (2007) Iron-binding properties of plant phenolics and cranberry’s bio-effects. Dalton Trans (43): 4951 – 4961

In conclusion, our results suggest that a decrease in cytokine concentrations during high flavonol consumption may serve as a risk indicator for colorectal cancer prevention. Verification of these results in other prospective cohorts with high quality and repeated dietary and serum cytokine measures is needed to clarify the role of serum cytokines as indicators of a chemopreventive response to dietary flavonols.

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Conflict of interest

The authors declare no conflict of interest.

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low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 342: 1149–1155
Schetter AJ, Nguyen GH, Bowman ED, Mathe EA, Yuen ST, Hawkes JE, Croce CM, Leung SY, Harris CC (2009) Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. Clin Cancer Res 15: 5878–5887
Si D, Wang Y, Zhou YH, Guo Y, Wang J, Zhou H, Li ZS, Fawcett JP (2009) Mechanism of CYP2C9 inhibition by flavones and flavonols. Drug Metab Dispos 37: 629–634
Stanilov N, Miteva L, Mintchev N, Stanilova S (2009) High expression of Foxp3, IL-23p19 and survivin mRNA in colorectal carcinoma. Int J Colorectal Dis 24: 151–157
Theodoratou E, Kyle J, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R, Porteous M, Dunlop M, Campbell H (2007) Dietary flavonoids and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 16: 684–693
Tiong KH, Yiap BC, Tan EL, Ismail R, Ong CE (2010) USDA database for the naturally occurring flavonoids on cytochrome P450 2A6 (CYP2A6) activity. Xenobiotica 40: 458–466.
U.S. Department of Agriculture ARS (2007) USDA database for the flavonoid content of selected foods. In Nutrient Data Laboratory web site: USDA.

Appendix

The members of the Polyp Prevention Study Group participated in the conduct of the Polyp Prevention Trial. However, the data presented in this manuscript and the conclusions drawn from them are solely the responsibility of the above listed co-authors. National Cancer Institute—Schatzkin A, Lanza E, Cross AJ, Corle D, Freedman LS, Clifford C, Tangrea J; Bowman Gray School of Medicine—Cooper MR, Paskett E (currently Ohio State University), Quandt S, DeGraffinreid C, Bradham K, Kent L, Self M, Boyles D, West D, Martin L, Taylor N, Dickenson E, Kuhn P, Harmon J, Richardson I, Lee H, Marceau E; University of New York at Buffalo—Lance MP (currently University of Arizona), Marshall JR (currently Roswell Park Cancer Center), Hayes D, Phillips J, Petrelli N, Shelton S, Randall E, Blake A, Wodarski L, Deinzer M, Melton R; Edwards Hines, Jr Hospital, Veterans Administration Medical Center—Iber FL, Murphy P, Bote EC, Brandt-Whittington L, Haroon N, Kazi N, Moore MA, Orloff SB, Ottosen WJ, Patel M, Rothschild RL, Ryan M, Sullivan JM, Verma A; Kaiser Foundation Research Institute—Caan B, Selby JV, Friedman G, Lawson M, Taft G, Snow D, Belfay M, Schoenberger M, Sampel K, Giboney T, Uronis JM, Muhlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C (2009) Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One 4: e6026
Vijayababu MR, Arunkumar A, Kanagaraj P, Venkataraman P, Krishnamoorthi G, Arunakaran J (2006) Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). Mol Cell Biochem 287: 109–116
Wang L, Tu YC, Lian TW, Hung TT, Yen JH, Wu MJ (2006) Distinctive antioxidant and antiinflammatory effects of flavonoids. J Agric Food Chem 54: 9798–9804
Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett 269: 281–290
Wilms LC, Hollman PC, Boots AW, Kleinjans JC (2005) Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutat Res 582: 155–162
Yoon JH, Baek SJ (2005) Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med J 46: 585–596
Zhang W, Zhang F (2009) Effects of quercetin on proliferation, apoptosis, adhesion and migration, and invasion of HeLa cells. Eur J Gynaecol Oncol 30: 60–64
Randel M; Memorial Sloan-Kettering Cancer Center—Shike M, Winawer S, Bloch A, Mayer J, Morse R, Latkany L, D’Amato D, Schaffer A, Cohen L; University of Pittsburgh—Weissfeld J, Schoen R, Schade RR, Kuller L, Gahagan B, Caggiula A, Lucas C, Coyne T, Pappert S, Robinson R, Landis V, Misko S, Search L; University of Utah—Burt RW, Slattery M, Viscofsky N, Benson J, Neilson J, McDivism R, Briley M, Heinrich K, Samowitz W; Walter Reed Army Medical Center—Kikendall JW, Mateski DJ, Wong R, Stoute E, Jones-Miskovsky V, Greaser A, Hancock S, Chandler S; Data and Nutrition Coordinating Center (West)—Cahill J, Hasson M, Daston C, Brewer B, Zimmerman T, Sharbaugh C, O’Brien B, Cranston L, Odaka N, Umbel K, Pinsky J, Price H, Slomim A; Central Pathologists—Lewin K (University of California, Los Angeles), Appelhan H (University of Michigan); Laboratories—Bachorik PS, Lovejoy K (Johns Hopkins University); Sowell A (Centers for Disease Control); Data and Safety Monitoring Committee—Greenberg ER (chair) (Dartmouth University); Feldman E (Augusta, Georgia); Garza C (Cornell University); Summers R (University of Iowa); Weiland S (through June 1995) (University of Minnesota); DeMets D (beginning July 1995) (University of Wisconsin).