

# Associations of Coffee Drinking with Systemic Immune and Inflammatory Markers

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## Abstract

**Background:** Coffee drinking has been inversely associated with mortality as well as cancers of the endometrium, colon, skin, prostate, and liver. Improved insulin sensitivity and reduced inflammation are among the hypothesized mechanisms by which coffee drinking may affect cancer risk; however, associations between coffee drinking and systemic levels of immune and inflammatory markers have not been well characterized.

**Methods:** We used Luminex bead-based assays to measure serum levels of 77 immune and inflammatory markers in 1,728 older non-Hispanic Whites. Usual coffee intake was self-reported using a food frequency questionnaire. We used weighted multivariable logistic regression models to examine associations between coffee and dichotomized marker levels. We conducted statistical trend tests by modeling the median value of each coffee category and applied a 20% false discovery rate criterion to *P* values.

**Results:** Ten of the 77 markers were nominally associated (*P* trend < 0.05) with coffee drinking. Five markers withstood correction for multiple comparisons and included aspects of the host response namely chemotaxis of monocytes/macrophages (IFN $\gamma$ , CX3CL1/fractalkine, CCL4/MIP-1 $\beta$ ), proinflammatory cytokines (sTNFR $\text{II}$ ), and regulators of cell growth (FGF-2). Heavy coffee drinkers had lower circulating levels of IFN $\gamma$  [odds ratios (OR), 0.35; 95% confidence intervals (CI), 0.16–0.75], CX3CL1/fractalkine (OR, 0.25; 95% CI, 0.10–0.64), CCL4/MIP-1 $\beta$  (OR, 0.48; 95% CI, 0.24–0.99), FGF-2 (OR, 0.62; 95% CI, 0.28–1.38), and sTNFR $\text{II}$  (OR, 0.34; 95% CI, 0.15–0.79) than non-coffee drinkers.

**Conclusions:** Lower circulating levels of inflammatory markers among coffee drinkers may partially mediate previously observed associations of coffee with cancer and other chronic diseases.

**Impact:** Validation studies, ideally controlled feeding trials, are needed to confirm these associations. *Cancer Epidemiol Biomarkers Prev*; 24(7); 1052–60. ©2015 AACR.

## Introduction

Coffee is a common dietary exposure that may favorably impact human health. According to a recent industry report, more than 60% of U.S. adults drink coffee daily (1). Coffee drinking appears to be inversely associated with total mortality (2–4) as well as cancers of the endometrium (5), colon (6, 7) and liver (8), fatal prostate cancer (9), type II diabetes (10), and chronic liver disease (11). Mechanisms underlying these observed associations are poorly understood, but improved insulin sensitivity (12–15)

and reduced inflammation (16–21) are suggested pathways by which coffee consumption may alter risk of certain cancers and related chronic diseases. For example, coffee is a rich source of polyphenols, and evidence suggests that polyphenols may impact immune function and chronic inflammation (22). Prior studies of coffee drinking and systemic inflammation have considered a small number of select markers, such as C-reactive protein (CRP) and interleukin-6 (IL6), and are marked by inconsistent results (16–21, 23). Thus, associations between coffee drinking and levels of immune and inflammatory markers in the blood have not yet been well characterized.

A broad study of immune and inflammatory markers in relation to coffee drinking may provide insight into key markers that should be measured in prospective studies that aim to identify the mechanisms mediating observed associations between coffee drinking and disease. In the current study, we used data from 1,728 individuals who participated in the screening arm of the population-based Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial to explore associations between coffee drinking and variation in the systemic levels of 77 inflammatory and immune markers.

## Materials and Methods

### The PLCO Cancer Screening Trial

The PLCO Cancer Screening Trial is a multicenter randomized screening trial of prostate, lung, colorectal, and ovarian cancer that enrolled approximately 155,000 adults, ages 55 to 74 years, from

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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the general U.S. population between 1993 and 2001 (24, 25). Questionnaires were administered to study participants at baseline to collect data on demographics, diet, and other general risk factors for disease (25). Annual health questionnaires and linkage to the National Death Index were used to ascertain incident cancer cases, which were subsequently confirmed by medical chart review (24). The PLCO Cancer Screening Trial was approved by the Institutional Review Board at the National Cancer Institute and at the 10 participating study centers; all participants gave informed consent.

### Study population

The present cross-sectional investigation includes participants from the screening arm of PLCO who were selected as either cases or controls for one of three previously conducted nested case-control studies of lung cancer (526 cases and 592 controls; ref. 26), ovarian cancer (150 cases and 149 controls; ref. 27), or non-Hodgkin lymphoma (NHL; 301 cases and 301 controls; ref. 28). Participants in the three case-control studies were weighted to the screening-arm of PLCO using weights discussed in *Statistical analysis*. Detailed descriptions of the exclusion criteria, matching factors, and inflammatory markers measured in these case-control studies have been reported elsewhere (29). Six individuals were included in two or more studies but were counted only once, for the case-control study to which they were first selected, in this analysis. The combined dataset excluded individuals who reported a history of cancer before baseline ( $n = 31$ ), incomplete smoking data ( $n = 11$ ), or a race other than non-Hispanic White ( $n = 149$ ). An insufficient number of individuals in other racial/ethnic groups precluded the inclusion of race in the model that was used to calculate study weights; consequently, this analysis only weights up to the non-Hispanic White population in the PLCO screening arm. For the present analysis, we additionally excluded individuals who lacked information on coffee consumption ( $n = 90$ ) resulting in an analytic sample size of 1,728. We included incident cancer cases as part of the main analysis as their blood was obtained before cancer diagnosis. After weighting, cancer cases accounted for a small fraction (2.8%) of the data, and the main findings were not meaningfully altered when we excluded cases from the primary analysis (Supplementary Table S1).

### Laboratory analysis

Serum samples from the lung cancer and NHL studies were collected at baseline; serum samples from the ovarian cancer study were collected at baseline or at a follow-up visit to ensure a relatively equal distribution of specimens between 2 and 14 years before diagnosis (27). All samples were centrifuged at  $1,200 \times g$  for 15 minutes, frozen within 2 hours of collection and stored at  $-70^\circ\text{C}$ . These samples were later used to measure circulating levels of 86 inflammatory and immune markers (Supplementary Table S2) that were selected based on the results of a methodologic study that assessed the performance and reproducibility of the multiplexed assays (30). The lung cancer study, NHL study, and ovarian cancer study measured 77, 83, and 60 markers, respectively (Millipore Inc.; Supplementary Table S3). Detailed descriptions of laboratory methods and assay reproducibility have been previously reported (26–28, 30). In brief, marker concentrations were calculated using either a four- or five-parameter standard curve. Serum samples were assayed in duplicate and averaged. To evaluate assay reproducibility coefficients-of-variation

(CV) and intraclass correlation coefficients (ICC) of log-transformed marker values were calculated from blinded duplicates in the lung and NHL studies and from duplicate measurements on study participants in the ovarian cancer study. Log-transformed ICCs were greater than 0.8 in 91%, 91%, and 78% of evaluable markers in the lung, NHL, and ovary studies, respectively (29). ICCs below 0.70 were reported for one marker (IL2) in the lung study (26) and five markers (IFN $\gamma$ , IL1RA, PYY, sIL4R, and sVEGF-2) in the ovarian cancer study (27). Additional information on quality control measurements can be found elsewhere (26–28). Eight markers with >90% of concentrations below the lowest limit of detection (LLOD) and one marker with >70% of concentrations above the upper limit of detection were excluded from the study, leaving 77 evaluable markers in our primary analysis.

### Statistical analysis

Propensity-score adjusted sampling weights were developed to ensure that analyses accounted for the study-specific sampling strategy and inclusion/exclusion criteria (Supplementary Table S3). These sampling weights, which have been described in detail elsewhere, allowed us to include all participants with biomarker data, including cancer cases who were cancer-free at the time of blood draw, and made our analysis as representative as possible of the non-Hispanic White PLCO screening arm population. Details on the development of sampling weights have been described in detail elsewhere (29). To summarize, study-, gender-, and case-control status-specific logistic regression models adjusted for age, detailed smoking history, and vital status on December 31, 2009 were used to estimate the probability that an eligible screening arm participant would be selected into any given case-control study. Combinations of study-specific weights were created for markers that were measured in multiple case-control studies (e.g., all three studies, lung and NHL, lung and ovary, and NHL and ovary; ref. 29).

Usual coffee consumption in the previous 12 months was assessed at baseline in the screening arm using a semi-quantitative 137-item food frequency questionnaire, which was based on two previously validated food frequency questionnaires (31, 32). A single coffee question queried about frequency (never to 6 or more times/day) and portion size of total, caffeinated or decaffeinated, coffee intake. Previous longitudinal studies have demonstrated that semi-quantitative food frequency questionnaires provide a reproducible and valid measure of coffee intake (33, 34), and in subgroup of PLCO participants who completed two food frequency questionnaires, approximately 3 years apart, the Spearman correlation coefficient was 0.76. Among coffee drinkers, the reported frequency of total coffee intake (less than once/month to 6 or more times/day) was multiplied by a gram amount, which was dependent on the gender of the subject and their response to serving size (small, medium, or large cup); gram amounts came from the USDA's 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII) database (35). Gram amounts were converted to number of medium (i.e., 12 oz.) cups for ease of interpretation. We then categorized individuals as either non-, moderate (below median coffee intake; <2.5 cups/day), or heavy (above median;  $\geq 2.5$  cups/day) coffee drinkers.

A considerable fraction of marker values below the LLOD (Supplementary Table S4) precluded analysis of all markers as continuous outcomes; therefore, markers were dichotomized as above or below the median value, or as detectable or undetectable

**Table 1.** Participant characteristics in (i) subjects with DQX and measured inflammatory marker data ( $N = 1,728$ ), (ii) weighted sample with DQX, and (iii) eligible PLCO Cancer Screening Trial sample

Characteristic	N (%) <sup>a</sup>	Weighted % <sup>a</sup>	PLCO screening arm, % <sup>a</sup>
Male	953 (55.2)	51.5	51.9
Age group (y)			
≤59	368 (21.3)	31.8	33.4
60–64	505 (29.2)	34.4	31.3
65–69	518 (30.0)	19.5	22.4
≥70	337 (19.5)	14.3	12.9
Education			
11 years or less	142 (8.2)	6.5	6.1
12 years or completed high school	440 (25.5)	24.9	23.4
Some post high school education	586 (33.9)	33.9	34.0
College graduate or postgraduate	560 (32.4)	34.7	36.3
BMI category (kg/m <sup>2</sup> )			
<25	615 (35.6)	31.7	32.2
25–30	747 (43.2)	45.7	43.0
≥30	345 (20.0)	21.1	23.9
Missing	21 (1.2)	1.5	0.9
Coffee drinking status (cups/day)			
None	144 (8.3)	10.2	12.5
<2.5	719 (41.6)	46.3	45.9
≥2.5	865 (50.1)	43.6	41.6
Smoking status			
Never	520 (30.1)	46.0	47.3
Former	820 (47.5)	44.3	43.1
Current	388 (22.5)	9.6	9.6
Alcohol drinking status (drinks/day)			
None	260 (15.1)	17.0	17.5
<1	1,008 (58.3)	60.8	58.5
1–3	281 (16.3)	13.2	15.4
≥3	179 (10.4)	9.0	8.6
Multivitamin use <sup>b</sup>	855 (49.5)	52.4	51.1
NSAID use <sup>c</sup>	1,110 (64.2)	64.5	62.3
Original case-control study			
Lung cancer study	947 (54.8)	42.5	—
NHL study	550 (31.8)	41.9	—
Ovarian cancer study	231 (13.4)	15.6	—
Red meat intake (g/1,000 kcal/day) <sup>d</sup>	33.9 (21.7–48.5)	31.4 (20.9–45.9)	32.8 (20.9–48.2)
White meat intake (g/1,000 kcal/day) <sup>e</sup>	19.2 (12.1–30.4)	19.6 (12.3–30.5)	20.7 (12.8–32.4)
Fruit intake (cups/1,000 kcal/day)	1.0 (0.6–1.5)	1.1 (0.7–1.6)	1.0 (0.7–1.5)
Vegetable intake (cups/1,000 kcal/day)	1.3 (0.9–1.6)	1.3 (1.0–1.7)	1.3 (1.0–1.6)

<sup>a</sup>Median and interquartile range (IQR) for continuous variables.

<sup>b</sup>Includes current and recent (within previous 2 years) multivitamin use.

<sup>c</sup>Includes aspirin and ibuprofen use.

<sup>d</sup>Includes red and processed meat.

<sup>e</sup>Includes poultry and fish.

if more than 50% of the values were below the LLOD. Models categorizing marker levels into quartiles, for markers with <25% below LLOD, or tertiles, for markers with 25%–50% below LLOD, produced similar results (data not shown).

First we assessed the associations between coffee drinking and each of the 77 dichotomized markers using weighted logistic regression models adjusted for the following potential confounders: age, sex, detailed smoking history, alcohol drinking, education, NSAID use, multivitamin use, body mass index (BMI), case-control study of origin, year of serum collection, estimated daily energy intake, and nutrient density adjusted daily intakes of fruit, vegetables, red meat, and white meat (e.g., poultry and fish). Less than 2% of the cohort lacked information on BMI; nonetheless, we included an indicator variable for missing BMI data in the regression models. All *a priori* selected covariates, except NSAID use, multivitamin use and food intake variables, altered risk estimates by 10% or more for some of the nominally significant markers. *P* values for trend across cate-

gories of coffee drinking were calculated by assigning each individual the midpoint value for the category and modeling this as a continuous variable. We applied a 20% false discovery rate (FDR) criterion (36) to the *P* values for trend from these models, and retained the markers that remained statistically significant for secondary analyses. We also considered a more stringent 5% FDR criterion. In addition, we conducted a sensitivity analysis that excluded markers with <25% detectability in any of the three nested case-control studies.

We used Pearson correlation coefficients to estimate the weighted correlations between the markers that met nominal criteria for significance (*P* for trend < 0.05) in the primary analysis for the lung study as this was the only study to include all 10 nominally significant markers. We also ran multivariable logistic regression models for each of the five inflammatory markers that met our 20% FDR criterion adjusted for the other four significant markers to evaluate the whether these associations were independent of each other.

**Table 2.** Participant characteristics<sup>a</sup> by level of coffee intake among non-Hispanic Whites in the screening arm of the PLCO Cancer Screening Trial

Characteristic	Coffee consumption			P <sup>b</sup>
	None (n = 144)	<2.5 cups/day (n = 719)	≥2.5 cups/day (n = 865)	
Sex				<0.0001
Female	70.3 (97)	67.6 (470)	23.1 (208)	
Male	29.7 (47)	32.2 (249)	76.9 (657)	
Age group (y)				0.51
≤59	31.2 (38)	31.2 (139)	32.6 (191)	
60–64	44.6 (46)	34.9 (219)	31.5 (240)	
65–69	11.4 (35)	19.3 (213)	21.6 (270)	
≥70	12.8 (25)	14.6 (148)	14.3 (164)	
Education				0.02
11 years or less	4.2 (8)	5.1 (54)	8.6 (80)	
12 years or completed high school	42.5 (51)	25.0 (181)	20.6 (208)	
Some post high school education	27.0 (44)	36.2 (254)	33.0 (288)	
College graduate or postgraduate	26.3 (41)	33.7 (230)	37.8 (289)	
BMI category (kg/m <sup>2</sup> ) <sup>c</sup>				0.11
<25	35.3 (55)	36.3 (274)	27.1 (286)	
25–30	45.7 (59)	40.3 (289)	53.0 (399)	
≥30	19.0 (29)	23.4 (146)	19.9 (170)	
Smoking status				<0.0001
Never	80.2 (92)	48.4 (276)	35.6 (152)	
Former	17.6 (40)	44.1 (332)	50.8 (448)	
Current	2.2 (12)	7.5 (111)	13.6 (265)	
Alcohol drinking status (drinks/day)				<0.0001
None	56.1 (64)	14.9 (86)	10.2 (110)	
<1	41.2 (64)	65.5 (467)	60.4 (477)	
1–3	2.2 (10)	12.9 (120)	16.1 (151)	
≥3	0.5 (6)	6.7 (46)	13.3 (127)	
Multivitamin use <sup>d</sup>	46.2 (71)	56.8 (391)	49.2 (393)	0.19
NSAID use <sup>e</sup>	57.8 (82)	62.9 (446)	67.9 (582)	0.33
Original case-control study				<0.0001
Lung cancer study	25.1 (49)	41.7 (347)	47.4 (551)	
NHL study	54.8 (56)	35.9 (222)	45.3 (272)	
Ovarian cancer study	20.1 (39)	22.4 (150)	7.3 (42)	
Red meat intake (g/1,000 kcal/day) <sup>f</sup>	22.9 (13.9–39.9)	30.3 (20.2–41.9)	35.4 (23.1–50.0)	<0.001
White meat intake (g/1,000 kcal/day) <sup>g</sup>	12.7 (7.0–20.2)	20.3 (13.7–33.0)	20.4 (12.2–29.7)	0.09
Fruit intake (cups/1,000 kcal/day)	1.5 (0.9–2.0)	1.2 (0.9–1.7)	0.9 (0.6–1.4)	<0.001
Vegetable intake (cups/1,000 kcal/day)	1.2 (1.0–1.7)	1.4 (1.0–1.8)	1.2 (0.9–1.5)	0.002

<sup>a</sup>Weighted column percent (unweighted *n*) for categorical variables and weighted median (IQR) for continuous variables.

<sup>b</sup>*P* value for Rao-Scott  $\chi^2$  test for categorical variables and Wald *F* test for continuous variables.

<sup>c</sup>*n* = 1,707 due to missing data on BMI.

<sup>d</sup>Includes current and recent (within previous 2 years) multivitamin use.

<sup>e</sup>Includes aspirin and ibuprofen use.

<sup>f</sup>Includes red and processed meat.

<sup>g</sup>Includes poultry and fish.

In secondary analyses, we stratified by sex and contributing study (lung, NHL, ovary) to explore possible effect modification. As smoking, diabetes, and BMI may be associated with both coffee drinking and chronic inflammation, we also performed sensitivity analyses in which we limited the analytic sample to never smokers (*n* = 520), those not reporting diabetes (*n* = 1,613), or participants with normal BMI (18.5 ≤ BMI < 25 kg/m<sup>2</sup>; *n* = 606). We formally tested for effect modification by sex, contributing study (lung, NHL, ovary), smoking status (never, former, current), diabetes status, and BMI (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m<sup>2</sup>) using the Wald  $\chi^2$  statistic to test the significance of the cross-product term for the single variable coffee and the variable of interest (e.g., sex) or to test the global significance of the cross-product terms for the single coffee variable and each level of the stratifying variable (e.g., smoking). All reported *P* values are based on two-sided tests. Finally, as a sensitivity analysis to our previously described trend analysis, we considered the natural log-transformed values of coffee intake plus a small constant [i.e.,

ln(coffee intake + *c*), where *c* = 0.01 cups] to assess the trend analysis for continuous coffee consumption. For secondary analyses, a *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc.); weighted analyses were conducted using SURVEY procedures in SAS.

## Results

Individuals with available immune and inflammatory markers were more likely to be current smokers and heavy coffee drinkers than the overall screening arm from which they were drawn (Table 1). This reflects the overrepresentation of smokers in the lung cancer study, as coffee drinking was associated with cigarette smoking in our study. Approximately, 62% and 50% of current and former smokers, respectively, were heavy coffee drinkers compared with 34% of never smokers. However, our weighted analytic sample was very similar to the eligible PLCO screening arm population with regard to smoking status and other

**Table 3.** ORs for high versus low levels<sup>a</sup> for 10 circulating inflammatory markers ( $P_{\text{trend}} < 0.05$ ) in three case-control studies nested within the PLCO Cancer Screening Trial ( $N = 1,728$ )

Inflammatory marker	Nondrinkers			Moderate coffee drinkers (<2.5 cups/day)			Heavy coffee drinkers (>2.5 cups/day)			$P_{\text{trend}}^c$
	OR	Low marker % (n) <sup>b</sup>	High marker % (n) <sup>b</sup>	OR (95% CI)	Low marker % (n) <sup>b</sup>	High marker % (n) <sup>b</sup>	OR (95% CI)	Low marker % (n) <sup>b</sup>	High marker % (n) <sup>b</sup>	
IFN $\gamma^d$	1.00	5.5 (83)	4.6 (61)	0.69 (0.33–1.43)	26.6 (418)	19.6 (301)	0.35 (0.16–0.75)	29.1 (465)	14.5 (400)	<b>0.0003</b>
CX3CL1/fractalkine <sup>d</sup>	1.00	8.0 (117)	2.2 (27)	0.46 (0.19–1.10)	38.3 (598)	7.9 (121)	0.25 (0.10–0.64)	39.1 (730)	4.5 (135)	<b>0.0032</b>
CCL4/MIP-1 $\beta$	1.00	4.4 (69)	5.8 (75)	0.85 (0.44–1.65)	20.9 (348)	25.4 (371)	0.48 (0.24–0.99)	23.9 (452)	19.7 (413)	<b>0.0050</b>
FGF-2 <sup>d</sup>	1.00	7.3 (95)	2.9 (49)	1.21 (0.57–2.57)	31.1 (501)	15.1 (218)	0.62 (0.28–1.38)	34.1 (599)	9.5 (266)	<b>0.0080</b>
sTNFR $\text{II}^e$	1.00	2.9 (46)	7.2 (98)	0.52 (0.24–1.14)	22.7 (345)	23.6 (374)	0.34 (0.15–0.79)	23.1 (395)	20.4 (470)	<b>0.0112</b>
TGF $\alpha$	1.00	5.4 (76)	4.8 (68)	1.60 (0.79–3.26)	20.4 (328)	25.8 (391)	0.87 (0.41–1.84)	25.6 (397)	8.0 (468)	0.0189
TNF $\alpha$	1.00	3.2 (49)	6.9(95)	0.43 (0.22–0.83)	23.1 (328)	23.2 (391)	0.32 (0.16–0.65)	23.3 (408)	20.3 (457)	0.0261
SAP <sup>e</sup>	1.00	4.1 (21)	2.3 (27)	1.78 (0.52–6.08)	22.0 (151)	27.6 (195)	0.75 (0.20–2.72)	22.8 (212)	21.2 (338)	0.0324
sTNFR $\text{I}^e$	1.00	4.7 (59)	5.5 (85)	0.97 (0.47–1.98)	21.7 (343)	24.5 (376)	0.61 (0.29–1.31)	22.9 (405)	20.7 (460)	0.0330
CCL19/MIP-3 $\beta^f$	1.00	5.0 (50)	5.6 (55)	1.06 (0.47–2.41)	20.6 (265)	25.4 (304)	0.64 (0.28–1.48)	23.5 (424)	20.0 (399)	0.0340

NOTE: Values in bold indicate significant  $P$  values after 0.20 false discovery rate (FDR) correction.

<sup>a</sup>ORs were estimated with weighted logistic regression. All models adjusted for NSAID use (yes or no); multivitamin use (yes or no); BMI category (0–25 kg/m<sup>2</sup>, 25–30 kg/m<sup>2</sup>, or  $\geq 30$  kg/m<sup>2</sup>); smoking status (no tobacco use, current smoker, quit 0–<10 y ago, quit 10 to <20 y ago, quit 20 to <30 y ago, quit 30 to <40 y ago, quit  $\geq 40$  y ago, or never smoked cigarettes but smoke pipe/cigars); age ( $\leq 59$  y, 60–64 y, 65–69 y, or 70+ y); alcohol drinking (none, <1 drink/day, 1–3 drinks/day, or >3 drinks/day); education ( $\leq 11$  y, 12 y or completed high school, some post high school education, or college graduate or post-graduate); sex (male or female); case-control study of origin (lung, NHL, or ovary); year of serum collection (calendar year); daily fruit intake (cups/1000 kcal/day); daily vegetable intake (cups/1000 kcal/day); daily red meat intake (g/1000 kcal/day); daily white meat (i.e., poultry and fish) intake (g/1000 kcal/day); and estimated daily energy intake (kcal/day). Low marker was defined as below the median marker level or below the limit of detection (LOD) in the case of an analyte that is detectable in less than 50% of samples; high marker was defined as equal to or above the median marker level or detectable in the case of an analyte that is detectable in less than 50% of the samples.

<sup>b</sup>Weighted row % (unweighted  $n$ ).

<sup>c</sup>Trend analysis treated coffee drinking as a single variable using the median value for each category of intake.

<sup>d</sup>Analyte detectable in less than 50% of samples.

<sup>e</sup> $n = 944$ , marker not measured in NHL and ovarian cancer studies.

<sup>f</sup> $n = 1,497$ , marker not measured in ovarian cancer case-control study.

exposures, indicating that these weights help account for the nonrepresentative sampling.

Heavy coffee drinking appeared to be associated with male gender, heavier alcohol use, higher red meat intake as well as lower fruit and vegetable intake (Table 2). Coffee consumption did not differ meaningfully by age group, NSAID use, multivitamin use, BMI category, or white meat intake (Table 2).

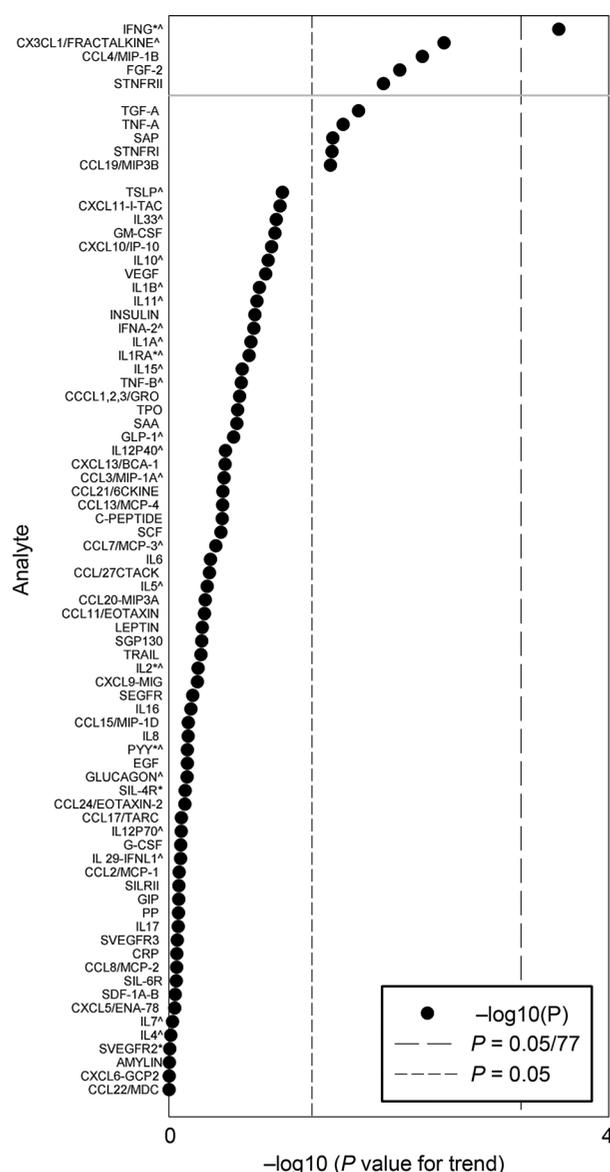
Ten of the 77 markers studied were nominally associated ( $P$  for trend < 0.05) with coffee drinking (Table 3) based on testing for trend in association across three levels of coffee consumption. Five markers were considered significant using a 20% FDR correction for multiple comparisons, and one marker, IFN $\gamma$ , was considered significant using a 5% FDR correction which was equivalent to the 5% Bonferroni corrected  $P$  value (i.e., 0.05/77; Fig. 1). These five markers include various aspects of the immune and inflammatory response namely chemotaxis of monocytes/macrophages (IFN $\gamma$ , CX3CL1/fractalkine, CCL4/MIP-1 $\beta$ ), proinflammatory cytokines (sTNFR $\text{II}$ ) and regulators of cell growth (FGF-2), and were inversely associated with ORs ranging from 0.25 to 0.87 for heavy versus non-coffee drinkers (Table 3). Associations appeared monotonic for IFN $\gamma$ , CX3CL1/fractalkine, CCL4/MIP-1 $\beta$ , and sTNFR $\text{II}$  but not for FGF-2. After excluding markers, including IFN $\gamma$  and CX3CL1/fractalkine, with <25% detectability in any of the three nested case-control studies, 53 markers remained; of the eight markers that reached nominal significance, one, sTNFR $\text{II}$ , met the 20% FDR correction (Supplementary Table S5).

These five markers were modestly to weakly correlated; weighted Pearson correlation coefficients ranged from –0.054 for IFN $\gamma$  and sTNFR $\text{I}$  to 0.65 for sTNFR $\text{II}$  and sTNFR $\text{I}$  (Supplementary Table S6), and associations of coffee drinking with IFN $\gamma$ , CCL4/MIP-1 $\beta$ , and sTNFR $\text{II}$  remained nominally significant following simultaneous adjustment for the other four markers

(Supplementary Table S7). Additional analyses confirmed that our conclusions were unaltered when evaluating marker levels in quartiles (markers with >75% detection) or tertiles (markers with 50%–75% detection; data not shown). Finally, the magnitude and direction of the associations were similar when we modeled coffee intake as a continuous variable  $\ln(\text{coffee intake} + c)$  (data not shown).

Sensitivity analyses revealed that associations were generally consistent across case-control study of origin (Supplementary Table S8). Tests for effect modification by sex (data not shown), smoking status, diabetes status, and BMI (Table 4) suggest that the associations are generally consistent across strata of sex, smoking, diabetes, and BMI ( $P_{\text{heterogeneity}} \geq 0.10$ ). However, associations of coffee drinking with levels of CCL4/MIP-1 $\beta$  ( $P_{\text{heterogeneity}} = 0.006$ ) and sTNFR $\text{II}$  ( $P_{\text{heterogeneity}} = 0.058$ ) were stronger in women than in men, although in the same general direction for both sexes. In addition, associations with IFN $\gamma$  ( $P_{\text{heterogeneity}} = 0.043$ ), CX3CL1/fractalkine ( $P_{\text{heterogeneity}} = 0.015$ ) and CCL4/MIP-1 $\beta$  ( $P_{\text{heterogeneity}} = 0.064$ ) appeared to be stronger in never-smokers than ever-smokers, although again of similar direction in both groups. In addition, the association with sTNFR $\text{II}$  was observed among those without self-reported diabetes only ( $P_{\text{heterogeneity}} = 0.001$ ).

Inflammatory markers, including CRP and IL6, that have been previously studied in relation to coffee drinking were not statistically significant overall or among nonsmokers (data not shown); however, heavy coffee drinking was significantly associated with lower circulating levels of insulin among nonsmokers only (OR, 0.30; 95% CI, 0.10–0.96;  $P = 0.03$ ; data not in table). Of the markers that were considered herein and statistically significantly associated with coffee intake in other population studies, CRP, IL6, SAA, and insulin, but not leptin, were statistically significantly ( $P < 0.05$ ) positively correlated with at least one of the five primary markers of interest. Weighted



**Figure 1.**

Summary of  $P$  values for trend from tests of multivariable-adjusted association with coffee drinking for all analytes included in the analysis ( $N = 77$  markers).  $P$  values for trend for analytes detectable in less than 50% of samples computed using Wald statistic of regression model parameters for dichotomized analyte level (undetectable vs. detectable).  $P$  values for trend for all other analytes were computed using Wald statistic of regression model parameters for dichotomized analyte level (<median vs.  $\geq$  median). All regression models were estimated with weighted logistic regression. Analytes with log-transformed intraclass correlations <70% in at least one of the three nested case-control studies are marked by an asterisk. Analytes with <25% detectability in at least one of the three nested case-control studies are marked by a caret. Analytes above the gray solid line met a 20% FDR criterion.

Pearson correlation coefficients for these previously identified markers and the current ones were generally weak and ranged from 0.08 for IFN $\gamma$  and SAA to 0.27 for sTNFR2 and CRP (data not in table). Adjustment for CRP, IL6, or SAA considerably attenuated the inverse association between coffee intake and

sTNFR2, although associations for other identified markers were unaffected (data not shown).

## Discussion

In the most comprehensive study of coffee drinking and systemic markers of inflammation and immunity to date, we found that heavier coffee consumption was associated with lower levels of a number of different inflammatory markers, including chemokines (CX3CL1/fractalkine, CCL4/MIP-1 $\beta$ ), cytokines (IFN $\gamma$ , sTNFR2), and basic fibroblast growth factor (FGF-2). Our findings were generally consistent among never and ever smokers, among those within the normal BMI range, and among those not reporting diabetes suggesting that confounding by these risk factors, which have been associated with chronic inflammation, is not a likely explanation for our findings.

Prior studies of coffee drinking and systemic inflammation have focused on a smaller group of inflammatory markers, and the results have been inconsistent. Ten of the previously examined inflammatory markers overlap with the 77 markers that were evaluated in our study (16–21, 23). We observed a similar inverse association between coffee consumption and sTNFR2 as reported in the Nurses' Health Study (18). We also found null associations for coffee drinking and four markers [monocyte chemoattractant protein-1 (CCL2/MCP-1), IL1 $\beta$ , IL1ra, and insulin] consistent with the results from prior studies (17, 20, 21). Our null results for CRP, leptin, IL6, serum amyloid-A (SAA), and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) are, however, consistent with some (17, 20, 21) but not all (16, 19, 20, 23) prior studies. Several observational studies have found inverse associations between coffee drinking and CRP in women (16, 18–20) and men (20). In contrast, a Greek study observed associations between moderate to heavy coffee consumption and higher levels of CRP as well as higher levels of IL6, SAA, and TNF $\alpha$  (23). In addition to chance, one possible explanation for the discrepant results between this study and our own is differences in coffee preparation method. In the Greek study, 88% of men and 92% of women reported drinking Greek-style unfiltered coffee.

Evidence from feeding studies suggests that the lipid-enriched fraction containing cafestol, which is present at markedly higher concentrations in unfiltered coffee, has a hypercholesterolemic effect (37) and may contribute to endothelial dysfunction (38). However, two small feeding trials of paper filtered coffee and systemic inflammation did not find associations with CRP (17, 21), IL6 (17, 21), leptin (17), SAA (17), or TNF $\alpha$  (21), as in our study. Nevertheless, additional observational studies with detailed information on coffee preparation and feeding trials with a range of coffee preparations are needed to examine this hypothesis.

Historically, coffee was regarded as a cardiovascular risk factor owing to findings from case-control studies (39, 40). Prospective cohort studies, which are less prone to selection bias and recall bias, have largely failed to reproduce these findings (41). In contrast, the more recent epidemiologic evidence suggests inverse associations with cardiovascular disease (41), total and cardiovascular disease mortality (2–4) as well as type II diabetes (10), Parkinson's disease (42), chronic liver disease (11), and certain cancers (5, 6, 8). One hypothesis, which may explain the numerous associations of coffee drinking with health outcomes, is that constituents of coffee, such as polyphenols, have

**Table 4.** ORs for high versus low levels<sup>a</sup> for the top five circulating inflammatory markers in three case-control studies nested within the PLCO Cancer Screening Trial among never smokers, individuals without diabetes, normal BMI (18.5–25 kg/m<sup>2</sup>)

Inflammatory marker	Subgroup	Coffee drinking status			<i>P</i> <sub>trend</sub>	<i>P</i> <sub>heterogeneity</sub> <sup>b</sup>
		Nondrinkers OR	Moderate coffee drinkers (<2.5 cups/day) OR (95% CI)	Heavy coffee drinkers (≥2.5 cups/day) OR (95% CI)		
IFN $\gamma$ <sup>c</sup>	Overall <sup>d</sup>	1.00	<b>0.69 (0.33–1.43)</b>	<b>0.35 (0.16–0.75)</b>	<b>0.0003</b>	—
	Never smokers <sup>e</sup>	1.00	0.63 (0.35–1.61)	0.19 (0.06–0.58)	0.0005	0.0434
	Diabetes-free <sup>f</sup>	1.00	0.76 (0.36–1.60)	0.38 (0.17–0.84)	0.0006	0.7554
	Normal BMI <sup>g</sup>	1.00	1.06 (0.32–3.47)	0.72 (0.20–2.54)	0.3019	0.4411
CX3CL1/fractalkine <sup>c</sup>	Overall <sup>d</sup>	1.00	<b>0.46 (0.19–1.10)</b>	<b>0.25 (0.10–0.64)</b>	<b>0.0032</b>	—
	Never smokers <sup>e</sup>	1.00	0.39 (0.13–1.19)	0.18 (0.04–0.71)	0.0443	0.0145
	Diabetes-free <sup>f</sup>	1.00	0.42 (0.17–1.06)	0.24 (0.09–0.65)	0.0073	0.9845
	Normal BMI <sup>g</sup>	1.00	2.08 (0.43–10.16)	1.13 (0.20–6.32)	0.3304	0.1140
CCL4/MIP-1 $\beta$	Overall <sup>d</sup>	1.00	<b>0.85 (0.44–1.65)</b>	<b>0.48 (0.24–0.99)</b>	<b>0.0050</b>	—
	Never smokers <sup>e</sup>	1.00	0.65 (0.27–1.56)	0.22 (0.08–0.67)	0.0033	0.0638
	Diabetes-free <sup>f</sup>	1.00	0.94 (0.48–1.85)	0.49 (0.24–1.02)	0.0034	0.9578
	Normal BMI <sup>g</sup>	1.00	1.05 (0.35–3.11)	0.48 (0.14–1.61)	0.3372	0.7972
FGF-2 <sup>c</sup>	Overall <sup>d</sup>	1.00	<b>1.21 (0.57–2.57)</b>	<b>0.62 (0.28–1.38)</b>	<b>0.0080</b>	—
	Never smokers <sup>e</sup>	1.00	0.83 (0.34–2.03)	0.32 (0.10–1.03)	0.0291	0.3399
	Diabetes-free <sup>f</sup>	1.00	1.14 (0.53–2.48)	0.54 (0.23–1.24)	0.0036	0.3721
	Normal BMI <sup>g</sup>	1.00	2.50 (0.56–11.14)	2.48 (0.54–11.35)	0.6746	0.3963
sTNFR $\text{II}$	Overall <sup>d</sup>	1.00	<b>0.52 (0.24–1.14)</b>	<b>0.34 (0.15–0.79)</b>	<b>0.0112</b>	—
	Never smokers <sup>e</sup>	1.00	0.38 (0.15–0.97)	0.25 (0.09–0.73)	0.0418	0.9181
	Diabetes-free <sup>f</sup>	1.00	0.52 (0.24–1.14)	0.38 (0.17–0.89)	0.0497	0.0008
	Normal BMI <sup>g</sup>	1.00	0.76 (0.22–2.60)	0.58 (0.16–2.29)	0.4104	0.3062

<sup>a</sup>Estimated with weighted logistic regression. All models adjusted for covariates in overall model<sup>d</sup> except BMI category.

<sup>b</sup>*P* value for Wald  $\chi^2$  test of global significance for the cross-product term(s) for each stratifying variable and coffee drinking; 21 individuals excluded from analysis of coffee<sup>a</sup>BMI category interaction due to missing BMI.

<sup>c</sup>Analytes detectable in less than 50% of samples.

<sup>d</sup>Estimated with weighted logistic regression. All models adjusted for NSAID use (yes or no); multivitamin use (yes or no); BMI category (0–25 kg/m<sup>2</sup>, 25–30 kg/m<sup>2</sup>, ≥30 kg/m<sup>2</sup>); smoking status (no tobacco use, current smoker, quit 0–<10 y ago, quit 10 to <20 y ago, quit 20 to <30 y ago, quit 30 to <40 y ago, quit ≥40 y ago, or never smoked cigarettes but smoke pipe/cigars); age (≤59 y, 60–64 y, 65–69 y, or 70+ y); alcohol drinking (none, <1 drink/day, 1–3 drinks/day, or >3 drinks/day); sex (male or female; lung and NHL studies only); year of serum collection (calendar year); education (≤11 y, 12 y or completed high school, some post high school education, or college graduate or post-graduate); daily fruit intake (cups/1,000 kcal/day); daily vegetable intake (cups/1,000 kcal/day); daily red meat intake (g/1,000 kcal/day); daily white meat (i.e., poultry and fish) intake (g/1,000 kcal/day); and estimated daily energy intake (kcal/day).

<sup>e</sup>Estimated with weighted logistic regression. All models adjusted for covariates in overall model<sup>d</sup> except smoking status.

<sup>f</sup>Estimated with weighted logistic regression. All models adjusted for covariates in overall model<sup>d</sup>; 4 individuals with missing diabetes status excluded.

immunomodulatory and antiinflammatory properties (22). For example, decaffeinated coffee consumption has been shown to reduce hepatic concentrations of IFN $\gamma$  and TNF $\alpha$  in an animal model of nonalcoholic steatohepatitis (NASH), which is marked by a chronic inflammatory state (43). Chlorogenic acid, a constituent of both caffeinated and decaffeinated coffee, has been shown to improve insulin resistance through reduced gluconeogenesis and inflammation in the liver and reduced glucose absorption in the gut (44), and caffeic acid, a metabolite of chlorogenic acid, has been shown to inhibit the TNF $\alpha$  induced inflammatory response in human endothelial cells (45). Intake of caffeine, a naturally occurring compound in coffee, has been shown to improve endothelial function and reduce inflammation in patients with and without coronary artery disease (46), and *in vitro* and *in vivo* studies suggest that methylxanthines, such as caffeine and theophylline, alter inflammatory and immune cell function at concentrations that are reflective of human exposure (47). More specifically, caffeine has been shown to decrease TNF $\alpha$  expression in blood cells (48) and theophylline, which is used to treat asthma and chronic obstructive pulmonary disease (COPD), is believed to exert some of its therapeutic effects by downregulating inflammation (49). Finally, kahweol, a coffee diterpene, has been shown to inhibit nuclear factor (NF)- $\kappa$ B (50), a protein complex that controls DNA transcription and directly regulates expression of cytokines/chemokines, growth factors, and immunoreceptors including CX3CL1/fractalkine (51, 52), CCL4/MIP-1 $\beta$  (53), and IFN $\gamma$  (54).

Stratified analyses and tests for heterogeneity suggest that associations between coffee drinking and inflammatory markers do not vary considerably by sex, smoking status, or diabetes status. In several instances, however, there was evidence of a quantitative interaction in which the association differed in magnitude but not direction across subgroups. For example, associations appeared to be stronger among women than men for CCL4/MIP-1 $\beta$ , among never smokers than ever smokers for IFN $\gamma$  and CX3CL1/fractalkine, and among those without diabetes than those with diabetes for sTNFR $\text{II}$ . Given the sizable number of statistical tests conducted in secondary analyses, these results may be due to chance and should be interpreted with caution.

Strengths of our study include its broad characterization of coffee drinking and variations in systemic immune and inflammatory markers measured using validated technology, a relatively large sample size, the use of innovative design methods to weight the analyses to the population-based screening arm of the PLCO cohort, and our consideration of multiple comparisons in the primary analysis (36). In addition, detailed adjustment for smoking and subgroup analyses in never smokers suggests that the observed associations are not explained by residual confounding by smoking; moreover, none of the statistically significant markers reported herein overlap with inflammatory and immune markers that were statistically significantly associated with smoking in this population (29). Limitations of our study include the cross-sectional design, which precludes evaluation of changes in marker

level over time, and the lack of detailed information on brewing method as well as whether coffee was caffeinated or decaffeinated. In addition, 24 of the 77 markers considered in this analysis had low detectability (<25%) in at least one of the three nested case-control studies. Future studies of these markers should consider more sensitive assays than the multiplex assays used herein. Our findings may not be generalizable to non-White or younger populations. Finally, in spite of a relatively large sample size, we had only modest statistical power to test interactions.

In conclusion, our study finds inverse associations between coffee drinking and systemic levels of a number of different inflammatory markers, including four markers (CX3CL1/fractalkine, CCL4/MIP-1 $\beta$ , IFN $\gamma$ , and FGF-2) that have not been previously considered in human studies of coffee drinking. Higher coffee drinking has been associated with lower risk of mortality (2–4) and numerous chronic diseases (5, 6, 8, 10, 42). If these associations were a result of coffee's effects on the immune system, then our data identify promising markers for future studies aiming to understand whether immune and inflammatory markers mediate the association between coffee and disease. Randomized feeding trials exploring the impact of coffee or coffee constituents, such as caffeine, on a broad array of inflammatory and immune markers are needed to validate the associations observed in our study. In addition, prospective-based studies (e.g., nested case-control studies in large cohorts) of coffee drinking and health that can measure variations in immune and inflammatory markers, including the markers examined herein, may provide insight into possible mediators of observed coffee-disease associations and ultimately elucidate the potentially etiologic role of coffee drinking in the development of disease.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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### Disclaimer

The opinions and conclusions expressed in this article are solely the views of the author(s) and do not necessarily reflect those of the Food and Drug Administration.

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