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FULL PAPER

BJC

British Journal of Cancer (2013) 108, 1891–1898 | doi: 10.1038/bjc.2013.172

Keywords: C-reactive protein; interleukin-6; soluble tumour necrosis factor receptor 2; colorectal cancer; body mass index

A prospective study of plasma inflammatory markers and risk of colorectal cancer in men

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Background: Chronic inflammation may mediate risk of colorectal cancer (CRC); however, the association between circulating inflammatory markers and risk of CRC has been inconsistent.

Methods: We prospectively evaluated the association of plasma C-reactive protein (CRP), interleukin-6 (IL-6), and the soluble tumour necrosis factor receptor 2 (sTNFR-2) with incident CRC among 274 cases and 532 matched controls nested in the Health Professionals Follow-up Study.

Results: Multivariate relative risk (RR) of CRC comparing the extreme quartiles of plasma IL-6 was 1.54 (95% confidence interval (CI), 0.99–2.40; $P_{trend} = 0.02$). However, after excluding cases diagnosed within 2 years of blood draw, this association was not statistically significant (RR = 1.26, 95% CI, 0.78–2.05; $P_{trend} = 0.21$). In analyses restricted to cases diagnosed at least 2 years after blood draw, the association of IL-6 with CRC appeared to differ by body mass index such that the significantly positive association was only present among lean individuals ($P_{interaction} = 0.03$). We did not observe any significant association between CRP or sTNFR-2 and CRC.

Conclusion: Plasma inflammatory markers are not generally associated with risk of CRC among men. However, the possibility that plasma IL-6 is associated with increased risk of CRC among lean men requires further investigation.

Chronic inflammation has an important role in colorectal cancer (CRC). Patients with inflammatory bowel disease have a 2- to 3-fold greater lifetime risk of developing CRC (Bernstein *et al*, 2001). Conversely, regular, long-term aspirin and nonsteroidal anti-inflammatory drug (NSAID) use has been shown to reduce CRC risk by 30–40% (Flossmann and Rothwell, 2007). Recent evidence also shows that chronic inflammation may partially underlie the causal link between obesity and colorectal neoplasia (Kant and Hull, 2011). Chronic inflammatory responses promote carcinogenesis by inducing DNA damage, suppressing DNA repair, stimulating angiogenesis and cell proliferation, and inhibiting apoptosis (Coussens and Werb, 2002).

Circulating inflammatory proteins, such as C-reactive protein (CRP), interleukin-6 (also known as IL-6) and tumour necrosis factor (TNF)-α, are frequently used as biomarkers of chronic inflammation and may mediate the inflammatory response (Libby and Ridker, 2004; Hussain and Harris, 2007). However, because inflammation is a component of the host response to cancer (Coussens and Werb, 2002), elevated inflammatory markers may result from occult cancer or a premalignant state rather than having a causal role in carcinogenesis (Allin and Nordestgaard, 2011).

Data on the association between baseline circulating inflammatory markers and risk of CRC has been inconsistent. Among 12 prospective studies, 5 showed a significant association between CRP and CRC (Erlinger *et al*, 2004; Il'yasova *et al*, 2005; Gunter *et al*, 2006; Otani *et al*, 2006; Aleksandrova *et al*, 2010), whereas the remaining studies reported generally null (Ito *et al*, 2005; Siemes *et al*, 2006; Trichopoulos *et al*, 2006; Allin *et al*, 2009; Heikkila *et al*, 2009; Chan *et al*, 2011) or even inverse associations (Zhang *et al*, 2005). The few studies examining IL-6 have not observed strong

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Received 1 February 2013; revised 20 March 2013; accepted 25 March 2013; published online 16 April 2013

relationships (Il'yasova *et al*, 2005; Heikkila *et al*, 2009; Chan *et al*, 2011; Ho *et al*, 2012). We recently reported plasma soluble tumour necrosis factor receptor 2 (sTNFR-2, also known as TNF receptor superfamily member 1B, TNFRSF1B), a surrogate marker for TNF-α (Diez-Ruiz *et al*, 1995; Pai *et al*, 2004), was significantly associated with increased risk of CRC in women (Chan *et al*, 2011), whereas other studies did not find any association between TNF-α and CRC in an elderly population (Il'yasova *et al*, 2005) or in postmenopausal women (Ho *et al*, 2012).

To extend these findings, we conducted a nested case-control study within the Health Professionals Follow-up Study (HPFS) to investigate the relationship of these plasma inflammatory markers with incidence of CRC among men over 13–15 years of follow-up. As these men have also provided detailed data on a range of exposures associated with inflammation, we also had the unique opportunity to prospectively evaluate the relationship between levels of inflammatory markers in relation to these lifestyle factors and risk of CRC.

MATERIALS AND METHODS

Study population. The HPFS is an ongoing prospective cohort that includes 51 529 US male health professionals, aged 40–75 years at enrolment, who returned a detailed survey about their lifestyle, medication use, medical diagnoses, and a semiquantitative food frequency questionnaire (FFQ) in 1986. With a follow-up rate exceeding 90%, men have returned biennial questionnaires to update lifestyle information and medical diagnoses; dietary information is updated every 4 years. More details regarding the main HPFS cohort have been published elsewhere (Rimm *et al*, 1991; Giovannucci *et al*, 1994). We requested written permission to acquire medical records and pathological reports from men who reported CRC. We identified deaths with over 96% sensitivity through the National Death Index and next-of-kin (Stampfer *et al*, 1984). For all deaths, we requested permission from next-of-kin to review medical records. A study physician, blinded to exposure information, reviewed records to confirm cases of CRC and to extract information on histological type, anatomic location, and stage of the cancer (Greene *et al*, 2002).

Between 1993 and 1995, 18 225 men returned a blood specimen on ice packs by overnight courier. Upon receipt, blood samples were immediately centrifuged, aliquoted into plasma, and stored in continuously monitored liquid nitrogen freezers ($-130\,^{\circ}C$ or below). More than 95% of samples arrived in our laboratory within 24 h of collection. Among these men who provided a plasma sample, we confirmed 287 incident CRC cases diagnosed between the date of blood draw and 1 January 2008. Using risk set sampling, we randomly selected two controls for each case matched on age (within 2 years) and month per year of blood donation from eligible participants who were alive and free of cancer (except for non-melanoma skin cancer) at the time of diagnosis of the case. We excluded 13 cases that failed laboratory assays. Among remaining cases, 16 were matched to only one control. Thus, a total of 274 case and 532 control participants were included in our analysis. This study was approved by the Human Subjects Committee of the Harvard School of Public Health and the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital, and all participants provided informed consent upon return of the questionnaires and blood specimens.

Laboratory assays. In a core laboratory facility, we used a highly sensitive immunoturbidimetric assay (Denka Seiken Co, Tokyo, Japan) to measure CRP levels, an ultra-sensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) to measure IL-6, and an enzyme-linked immunosorbent assay (R&D Systems) to measure sTNFR-2 levels. To examine the stability of plasma biomarkers according to our storage techniques, we previously compared the concentrations in samples taken from 17 female volunteers, which were processed and plasma frozen immediately after venipuncture (the standard processing methods), with samples that were stored as heparinised whole blood for 24–36 h before processing (mimicking our collection conditions). The mean IL-6, CRP, and sTNFR-2 values were almost identical and the intraclass correlations between results of the two collection methods were 0.99 for CRP, 0.93 for IL-6, and 0.91 for sTNFR-2, demonstrating that our collection methods did not adversely affect sample integrity (Pai *et al*, 2002; Chan *et al*, 2011). Samples from case patients and their matched control participants were analysed in the same batch. Quality control samples were randomly interspersed among the case-control samples. Personnel blinded to quality-control and case-control status conducted all assays. Interleukin-6 and sTNFR-2 were assayed in a single run, whereas CRP was measured in two batches over 6 years, and drift samples were included to assess laboratory drift over time. The intraclass correlation between results of the two drift batches was 0.9998, suggesting the extraordinary stability of plasma CRP over time. The intra-assay coefficients of variation from blinded quality control samples were 7.8% for CRP, 12.1% for IL-6, and 10.1% for sTNFR-2.

Questionnaire and dietary information. As in previous analyses (Chan *et al*, 2011), we used information collected from each biennial questionnaire on weight, multivitamin use, aspirin use, NSAID use, smoking, and endoscopic screening. Physical activity was calculated by summing the products of time spent on a variety of recreational or leisure-time activities with the average metabolic equivalent (MET) for that activity (Chasan-Taber *et al*, 1996). Dietary information was obtained from the 1986, 1990, and 1994 FFQs, and nutrient intake was adjusted for total energy consumption using the residual method (Willett *et al*, 1997). Family history of CRC was collected on the 1986, 1990, and 1992 questionnaires. In 1987, ~65% of participants provided measurements of hip and waist circumferences after receiving a supplementary questionnaire, which included a tape measure and illustrated instructions. We have previously shown that these self-reported measurements in our cohort compared with technician measurements are reasonably accurate (Pearson's correlation coefficient: for weight, $r=0.97$; for waist circumference, $r=0.95$; for hip circumference, $r=0.88$; for waist-to-hip ratio, $r=0.69$) (Rimm *et al*, 1990).

Statistical analysis. We compared means (± s.d.) with medians (± interquartile ranges) of continuous variables for case and control participants using paired *t* test and Wilcoxon-signed rank test, respectively. We used the conditional logistic regression to compare categorical variables.

We employed multivariate linear models to assess the associations between lifestyle factors and plasma biomarker levels. The logarithmic transformation of biomarker measurements was conducted to fit the normal assumption of the model. We also added a quadratic term of each continuous covariate to test for the possible nonlinear relationship with biomarkers, and the test for nonlinearity was not statistically significant. Therefore, we report results from models without quadratic terms.

We calculated quartile cutpoints for plasma inflammatory markers based on the distribution among control participants. Relative risks (RRs) and corresponding 95% confidence intervals (CIs) were estimated to assess associations between quartiles of biomarker levels and CRC using conditional logistic regression. Tests for trend were conducted using the median values for each quartile of each plasma biomarker as a continuous variable in the regression models. We also performed restricted cubic spline analysis to examine for a possible nonlinear relationship between each plasma inflammatory marker and CRC risk (Durrleman and Simon, 1989). We used stepwise selection to determine knots ($P<0.05$ for both stay and entry in the model) and tested for

nonlinearity using the likelihood ratio test by comparing the model with only the linear term to the model with the linear and the cubic spline terms.

In multivariate models, we additionally adjusted for family history of CRC, history of endoscopic screening, body mass index (BMI), physical activity (in MET-hours per week), pack-years of smoking, alcohol consumption, regular aspirin/NSAID use, regular use of multivitamins, red meat intake, and energy-adjusted daily intake of folate, calcium and total fibre. To better approximate long-term lifestyle and nutritional status in our analyses, we used cumulative average BMI, physical activity, aspirin/NSAID use and dietary intake variables derived from all biennial questionnaires beginning in 1986 through the time of blood collection. Missing information on a questionnaire was carried forward from available information on prior questionnaires.

To evaluate whether observed associations varied by risk factors for CRC, we conducted stratified analyses using unconditional logistic regression, additionally adjusting for the matching factors (that is, age and date of blood draw) in our multivariate models. To test for multiplicative interaction between stratification factors and biomarkers, we included cross-product terms for stratification factors and biomarkers to our models.

We also examined the possible heterogeneity in the associations between inflammatory markers and CRC, according to cancer subsite (proximal colon, distal colon, or rectum) and stage (I, II, III, or IV). A polytomous logistic regression model was used in which the association with biomarkers was allowed to vary between the case groups, but all the covariates were held constant. To calculate P difference between case groups, we performed a likelihood ratio test comparing the model described above with a model in which all the associations were held constant between case groups.

For sensitivity analyses, we used the extreme studentised deviate Many-Outlier procedure to identify outliers in each set of laboratory results (Rosner, 1983), and repeated above analyses after excluding outliers. We used SAS version 9.2 (SAS Institute, Inc, Cary, NC, USA) for all analyses with the exception of the polytomous logistic regression model, for which we used Stata version 11.0 (StataCorp, College Station, TX, USA). All statistical tests were two sided and $P<0.05$ was considered statistically significant.

RESULTS

Table 1 shows baseline characteristics of 274 CRC cases and 532 matched control participants. Overall, CRC cases were more likely to be obese and have family history of CRC, whereas control participants were more likely to have history of endoscopic screening. The median values of plasma CRP were 1.36 mg l^{-1} in cases and 1.11 mg l^{-1} in controls. For IL-6, the corresponding median values were 1.60 pg ml^{-1} among cases and 1.39 pg ml^{-1} among controls. For sTNFR-2, the corresponding median values were 2746 pg ml^{-1} among cases and 2725 pg ml^{-1} among controls. None of the three biomarkers appeared to significantly differ between cases and controls (for CRP, $P=0.72$; for IL-6, $P=0.42$; for sTNFR-2, $P=0.28$). The three markers were significantly correlated (Spearman's correlation coefficient: for CRP and IL-6, $r=0.50$; for CRP and sTNFR-2, $r=0.27$; for IL-6 and sTNFR-2, $r=0.32$; all P values <0.001).

We examined the multivariate-adjusted associations of key lifestyle factors with plasma inflammatory markers among control participants. As shown in Table 2, age, obesity, and smoking were positively associated with high levels of CRP, IL-6, and sTNFR-2. Abdominal adiposity as measured by waist circumference appeared to be more strongly associated with inflammatory markers than BMI. Physical activity was inversely associated with inflammatory marker levels, although only the association with sTNFR-2 attained statistical significance ($P=0.03$). Alcohol consumption was not significantly associated with inflammatory markers. Compared with non-users, regular users of aspirin or NSAIDs had higher levels of inflammatory cytokines.

Table 3 shows the relationship between quartiles of plasma inflammatory markers and risk of CRC. In unadjusted analyses, both CRP and IL-6 were significantly associated with increased risk

Table 1. Baseline characteristics of study participants

Baseline characteristics	Cases ($n=274$)	Controls ($n=532$)	P-value
Mean age at blood draw (s.d.), year	65.8 (8.3)	65.9 (8.3)	0.29
Non-white, no. (%)	17 (6.2)	31 (5.8)	0.83
CRC in a parent or sibling, no. (%)	54 (19.7)	73 (13.7)	0.03
History of previous endoscopy, no. (%)	154 (56.2)	358 (67.3)	0.002
Current multivitamin use, no. (%)	130 (47.5)	275 (51.7)	0.25
Regular aspirin use, no. (%)[a]	115 (42.0)	258 (48.5)	0.08
Regular NSAID use, no. (%)[b]	31 (11.3)	63 (11.8)	0.82
Current or past smoker, no. (%)[c]	152 (57.6)	273 (54.0)	0.34
Mean pack-years of smoking (s.d.)[d]	24.6 (17.9)	24.8 (18.9)	0.72
Mean BMI (s.d.), kg m^{-2}	26.2 (3.1)	25.3 (2.7)	<0.001
Mean waist circumference (s.d.), inch[e]	38.6 (3.5)	37.5 (3.3)	<0.001
Mean waist-to-hip ratio (s.d.)[e]	0.96 (0.05)	0.94 (0.05)	<0.001
Mean MET-hours (s.d.)	31.9 (26.9)	30.9 (25.1)	0.60
Mean daily intakes (s.d.)[f]			
Alcohol, g	12.2 (14.8)	11.9 (14.7)	0.77
Folate, μg	494 (209)	520 (228)	0.13
Calcium, mg	917 (385)	928 (343)	0.62
Red meat, servings	1.15 (0.77)	1.08 (0.69)	0.17
Total fiber, g	22.1 (6.4)	22.6 (6.5)	0.23
Median CRP (IQR), mg l^{-1}	1.36 (0.68–2.62)	1.11 (0.59–2.18)	0.72
Median IL-6 (IQR), pg ml^{-1}	1.60 (0.99–2.65)	1.39 (0.93–2.24)	0.42
Median sTNFR-2 (IQR), pg ml^{-1}	2746 (2346–3232)	2725 (2345–3336)	0.28

Abbreviations: BMI = body mass index; CRC = colorectal cancer; CRP = C-reactive protein; IL-6 = interleukin-6; IQR = inter-quartile range; MET = metabolic equivalent = (caloric need per kilogram body weight per hour activity)/(caloric need per kilogram body weight per hour at rest); NSAID = non-steroidal anti-inflammatory drug; sTNFR-2 = soluble tumour necrosis factor receptor 2.
[a]A standard tablet contains 325 mg aspirin, and regular users were defined as those who used at least two tablets per week.
[b]Regular users were defined as those who used at least two tablets per week.
[c]Thirty six participants with missing values were excluded from calculation.
[d]Never smokers ($N=345$) and participants with missing values ($N=45$) were excluded when calculating means and s.d.
[e]Measured in 1987, and 147 and 149 participants with missing information on waist circumference and waist-to-hip ratio were excluded from calculation of means and s.d., respectively.
[f]For dietary factors, up to 12 participants have missing information.

Table 2. Multivariate associations of lifestyle factors with levels of plasma inflammatory markers among control participants[a]

	CRP (mg l^{-1})		IL-6 (pg ml^{-1})		sTNFR-2 (pg ml^{-1})	
Variable	Relative change (%)[b]	*P*-value	Relative change (%)[b]	*P*-value	Relative change (%)[b]	*P*-value
Age, year	16.8	<0.001	18.3	<0.001	9.8	<0.001
BMI, kg m^{-2}	16.1	<0.001	4.8	0.11	1.7	0.13
Waist circumference, inch[c]	21.2	<0.001	7.2	0.04	2.2	0.08
Waist-to-hip ratio[c]	10.6	0.03	2.0	0.52	2.2	0.05
Physical activity, MET-hours per wk	−2.4	0.58	−4.4	0.12	−2.4	0.03
Pack-years of smoking	11.0	0.03	8.1	0.02	0.6	0.59
Alcohol consumption, g per day	3.0	0.54	2.6	0.42	−1.7	0.15
Regular aspirin/NSAID use	10.3	0.27	5.4	0.37	0.4	0.84

Abbreviations: BMI = body mass index; CRP = C-reactive protein; IL-6 = interleukin-6; MET = metabolic equivalent = (caloric need per kilogram body weight per hour activity)/(caloric need per kilogram body weight per hour at rest); NSAID = non-steroidal anti-inflammatory drug; sTNFR-2 = soluble tumour necrosis factor receptor 2.

[a]Multivariate linear models based on natural log-transformed biomarker levels included age (continuous, year), BMI (continuous, kg m^{-2}), physical activity(continuous, MET-hours per week), pack-years of smoking (continuous), alcohol consumption (continuous, g per day), regular aspirin/NSAID use (⩾2 tablets per week, yes or no), regular use of multivitamins (yes or no), and energy-adjusted intake of total fiber, saturated fat, monounsaturated fat, polyunsaturated fat and trans-fat (tertiles, g per day).

[b]Percentages of changes for one-s.d. increment of continuous variables (the s.d. of variables can be found in Table 1). For aspirin/NSAID use, percent of change of biomarker levels was calculated for regular users relative to non-users.

[c]Ninety one control participants with missing information on waist circumference and waist-to-hip ratio were excluded from the analysis, and BMI was excluded from the multivariate models.

of CRC. However, after adjustment for lifestyle and dietary risk factors, the magnitude of risk estimates were attenuated, and only IL-6 remained significantly associated with increased risk of CRC ($P_{trend} = 0.02$). Compared with men in the lowest quartile (Q1), men in the highest quartile (Q4) of plasma IL-6 had a multivariate RR of 1.54 (95% CI, 0.99–2.40). There was no association comparing extreme quartiles of CRP or sTNFR-2.

To assess the potential influence of subclinical neoplasia on circulating marker levels, we further stratified the analysis according to the follow-up time (within 2 years compared with after 2 years of blood draw, Table 3). Comparing extreme quartiles, we observed significant associations of CRP and IL-6 levels with CRC diagnosed within 2 years of blood draw (for CRP, $P_{trend} = 0.005$; for IL-6, $P_{trend} = 0.01$). In contrast, there was no significant association between CRP, IL-6, or sTNFR-2 and risk of CRC among case patients diagnosed after 2 years of follow-up (for CRP, $P_{trend} = 0.91$; for IL-6, $P_{trend} = 0.21$; for sTNFR-2, $P_{trend} = 0.83$). We also examined the possibility of a nonlinear relation between each plasma marker and risk of CRC using restricted cubic splines after excluding cases diagnosed within 2 years of follow-up. We did not find any nonlinear association for each marker (Supplementary Figures S1–3). We further excluded cases diagnosed within 6 years after blood collection, and the results did not materially change (for CRP, multivariate RR = 0.61, 95% CI, 0.30–1.23, $P_{trend} = 0.32$; for IL-6, multivariate RR = 1.32, 95% CI, 0.74–2.37, $P_{trend} = 0.22$; for sTNFR-2, multivariate RR = 0.74, 95% CI, 0.37–1.49, $P_{trend} = 0.44$). Thus, we excluded cases diagnosed within 2 years of follow-up for all subsequent analyses.

We then performed analyses of plasma inflammatory markers according to selected subgroups, including age at the time of blood collection, smoking, BMI, waist circumference, waist-to-hip ratio, physical activity, regular aspirin/NSAID use, and cancer subsite and stage. As shown in Table 4, the association of IL-6 with risk of CRC appeared significantly stronger in lean men (BMI <25 kg m^{-2}) than in men who were overweight or obese (BMI ⩾25 kg/m^2; $P_{interaction} = 0.03$). Among men with BMI <25 kg m^{-2}, men with high IL-6 levels (⩾median) had a significantly increased risk of CRC relative to men with low IL-6 levels (<median; multivariate RR = 1.76, 95% CI = 1.04–2.98). In contrast, among men with BMI ⩾25 kg m^{-2}, a nonsignificant inverse association was found between high IL-6 levels and cancer risk (multivariate RR = 0.74, 95% CI, 0.47–1.18). No significant interaction was identified for age, physical activity, or regular aspirin/NSAID use with inflammatory markers in relation to CRC risk. We also did not observe significant difference in the associations between markers and CRC risk according to subsite or stage (all *P* dif >0.05).

In sensitivity analysis, we additionally adjusted for race and time of the day when the blood sample was collected in our multivariate model, and the results remained essentially unchanged. We also repeated our analyses after excluding participants with outliers in laboratory measurements, or participants with history of coronary heart disease or diabetes mellitus. The results were not materially altered (data not shown).

DISCUSSION

In this prospective study of men, we showed that the significant associations between plasma CRP, IL-6 levels, and risk of CRC were restricted to cases diagnosed within 2 years of blood collection. After excluding cases that occurred within 2 years of follow-up, CRP, IL-6, or sTNFR-2 was not significantly associated with subsequent development of CRC.

Our results are supported by the findings of several large prospective studies that did not find any significant association between baseline circulating CRP (Ito *et al*, 2005; Zhang *et al*, 2005; Siemes *et al*, 2006; Trichopoulos *et al*, 2006; Allin *et al*, 2009; Heikkila *et al*, 2009; Chan *et al*, 2011), IL-6 (Il'yasova *et al*, 2005; Heikkila *et al*, 2009; Chan *et al*, 2011; Ho *et al*, 2012) or TNF-α (Il'yasova *et al*, 2005; Ho *et al*, 2012) levels and risk of CRC. In addition, a recent study that used instrumental variable analysis (Mendelian randomisation) also did not observe an association between either plasma CRP or genetically determined CRP levels and risk of CRC (Allin *et al*, 2010). A separate study demonstrated similar results in a large population (Siemes *et al*, 2006).

Our results differ from previous prospective studies that have shown significant associations of various inflammatory markers

Table 3. Relative risk of CRC according to plasma inflammatory markers

	Quartile[a]				
Analyte	**1**	**2**	**3**	**4**	P_{trend}[b]
CRP					
Median (mg l^{-1})	0.38	0.79	1.49	3.44	
No. of cases/controls	62/131	53/133	73/135	86/133	
Crude RR (95% CI)[c]	1.00 (reference)	0.86 (0.55–1.33)	1.14 (0.75–1.73)	1.38 (0.90–2.09)	0.04
Multivariate RR (95% CI)[d]	1.00 (reference)	0.80 (0.51–1.26)	1.07 (0.69–1.64)	1.24 (0.80–1.93)	0.11
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.78 (0.50–1.24)	0.91 (0.58–1.41)	1.17 (0.74–1.83)	0.18
Within 2 years of follow-up					
No. of cases/controls	7/24	3/11	8/20	24/27	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	1.18 (0.21–6.67)	1.04 (0.23–4.65)	4.73 (1.31–17.1)	0.005
After 2 years of follow-up					
No. of cases/controls	55/107	50/122	65/115	62/106	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.76 (0.47–1.23)	0.87 (0.53–1.40)	0.90 (0.54–1.52)	0.91
IL-6					
Median (pg ml^{-1})	0.73	1.15	1.64	3.42	
No. of cases/controls	54/133	57/132	74/134	89/133	
Crude RR (95% CI)[c]	1.00 (reference)	1.10 (0.71–1.73)	1.44 (0.93–2.23)	1.70 (1.12–2.59)	0.01
Multivariate RR (95% CI)[d]	1.00 (reference)	1.06 (0.67–1.68)	1.34 (0.85–2.11)	1.62 (1.05–2.50)	0.02
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.99 (0.61–1.59)	1.17 (0.74–1.87)	1.54 (0.99–2.40)	0.02
Within 2 years of follow-up					
No. of cases/controls	3/16	5/23	15/22	19/21	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	1.30 (0.22–7.65)	6.27 (1.21–32.63)	6.78 (1.36–33.8)	0.01
After 2 years of follow-up					
No. of cases/controls	51/117	52/109	59/112	70/112	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.96 (0.58–1.59)	0.94 (0.57–1.56)	1.26 (0.78–2.05)	0.21
sTNFR-2					
Median (pg ml^{-1})	2108	2535	2999	3765	
No. of cases/controls	68/133	66/133	78/133	62/133	
Crude RR (95% CI)[c]	1.00 (reference)	1.01 (0.66–1.53)	1.15 (0.76–1.76)	0.91 (0.58–1.45)	0.77
Multivariate RR (95% CI)[d]	1.00 (reference)	1.01 (0.66–1.56)	1.15 (0.74–1.79)	0.97 (0.60–1.57)	0.97
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.92 (0.59–1.43)	1.07 (0.68–1.68)	0.87 (0.54–1.43)	0.69
Within 2 years of follow-up					
No. of cases/controls	5/15	14/16	14/26	9/25	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	3.00 (0.67–13.48)	1.96 (0.45–8.61)	1.69 (0.29–9.77)	0.96
After 2 years of follow-up					
No. of cases/controls	63/118	52/117	64/107	53/108	
Multivariate RR (95% CI)[d] + BMI (kg m^{-2})	1.00 (reference)	0.80 (0.49–1.29)	1.00 (0.62–1.63)	0.89 (0.52–1.51)	0.83

Abbreviations: BMI = body mass index; CI = confidence interval; CRC = colorectal cancer; CRP = C-reactive protein; IL-6 = interleukin-6; RR = relative risk; sTNFR-2 = soluble tumour necrosis factor receptor 2.

[a]Quartiles of plasma inflammatory markers were based on the distribution among the control participants.

[b]Tests for linear trend were conducted using the median values for each quartile of analyte.

[c]Results were based on conditional logistic regression. Controls were matched to cases on age and date of blood collection.

[d]Results were based on conditional logistic regression (matched for age and date of blood collection) with adjustment for CRC in parent or sibling (yes or no), prior lower gastrointestinal endoscopy (yes or no), pack-years of smoking (continuous), alcohol consumption (continuous, g per day), physical activity (continuous, MET hours per week), regular aspirin/NSAID use (yes or no, ⩾2 tablets per week), regular use of multivitamins (yes or no), energy-adjusted intake of folate (including supplements, μg per day, continuous), calcium (including supplements, mg per day, continuous) and total fiber (continuous, g per day), and red meat intake (continuous, serving per day).

with risk of CRC (Erlinger *et al*, 2004; Il'yasova *et al*, 2005; Gunter *et al*, 2006; Otani *et al*, 2006; Aleksandrova *et al*, 2010; Chan *et al*, 2011). These inconsistent results could be due to several possible explanations. First, some results demonstrating an association between inflammatory markers and CRC may be related to reverse causation due to clinically undetected neoplasia at the time of blood collection. Tumour cells can produce inflammatory cytokines and induce surrounding tissue inflammation (Balkwill & Mantovani, 2001; Heikkila *et al*, 2007) and inflammatory markers can be indicators of the host immune response to tumour antigens (Coussens and Werb, 2002; Heikkila *et al*, 2007). Moreover, because colorectal carcinogenesis is characterised by a relatively slow and stepwise progression from normal to dysplastic epithelium to carcinoma, which may take years or even decades, studies of inflammatory markers in relation to incident CRC may be particularly susceptible to reverse causation due to occult neoplasia (Leslie *et al*, 2002). Similar to our results, several large prospective studies, including the CLUE II cohort (Erlinger *et al*, 2004), Women's Health Study (Zhang *et al*, 2005), and the Rotterdam Study (Siemes *et al*, 2006), have shown that exclusion of cases diagnosed in the first years of blood collection attenuated the associations between baseline CRP and subsequent development of CRC.

Second, inadequate control for potential confounders may influence the findings of prior studies. Obesity, physical activity,

Table 4. Relative risk of CRC according to plasma inflammatory markers by selected characteristics

	CRP			IL-6			sTNFR-2		
	No. of cases/controls			No. of cases/controls			No. of cases/controls		
	<1.11 mg l^{-1}	$\geqslant 1.11$ mg l^{-1}	RR (95% CI)	<1.39 pg ml^{-1}	$\geqslant 1.39$ pg ml^{-1}	RR (95% CI)	<2725 pg ml^{-1}	$\geqslant 2725$ pg ml^{-1}	RR (95% CI)
Age[a]									
<65 years	54/124	47/93	0.97 (0.57–1.64)	56/134	45/83	1.21 (0.71–2.04)	68/143	33/74	0.92 (0.52–1.61)
⩾65 years	51/140	80/175	0.99 (0.63–1.56)	47/131	84/184	1.07 (0.69–1.68)	47/123	84/192	1.12 (0.71–1.75)
Smoking[a]									
Never smokers	50/139	57/120	1.13 (0.70–1.84)	50/137	57/122	1.21 (0.74–1.97)	52/124	55/135	0.98 (0.60–1.60)
Ever smokers	55/125	70/148	0.82 (0.51–1.30)	53/128	72/145	0.99 (0.62–1.58)	63/142	62/131	0.93 (0.57–1.50)
BMI[a]									
<25 kg m^{-2}	48/145	38/117	0.86 (0.51–1.47)	36/145	50/117	1.76 (1.04–2.98)	40/135	46/127	1.31 (0.76–2.25)
$\geqslant 25$ kg m^{-2}	57/119	89/151	1.10 (0.71–1.71)	67/120	79/150	0.74 (0.47–1.18)	75/131	71/139	0.83 (0.53–1.30)
Waist circumference[a,b]									
<37 inch	32/119	24/80	1.09 (0.56–2.13)	25/110	31/89	1.99 (1.02–3.90)	31/105	25/94	0.99 (0.50–1.99)
⩾37 inch	50/100	77/142	0.89 (0.55–1.44)	55/107	72/135	0.86 (0.53–1.39)	60/114	67/128	1.01 (0.62–1.65)
Waist-to-hip ratio[a,b]									
<0.94	26/119	31/94	1.16 (0.61–2.23)	24/116	33/97	1.48 (0.76–2.89)	29/118	28/95	1.08 (0.55–2.14)
⩾0.94	56/100	69/128	0.91 (0.56–1.49)	56/101	69/127	0.92 (0.57–1.47)	61/101	64/127	0.91 (0.56–1.48)
Physical activity[a]									
<25 MET-hours per week	45/116	66/146	0.86 (0.52–1.43)	43/112	68/150	1.00 (0.61–1.64)	51/118	60/144	0.90 (0.55–1.49)
⩾25 MET-hours per week	60/148	61/122	1.12 (0.71–1.78)	60/153	61/117	1.23 (0.77–1.96)	64/148	57/122	1.14 (0.70–1.85)
Regular aspirin/NSAID use[a,c]									
Yes	53/139	62/149	0.90 (0.56–1.46)	52/139	63/149	0.93 (0.58–1.49)	48/134	67/154	1.17 (0.71–1.92)
No	52/125	65/119	1.11 (0.68–1.81)	51/126	66/118	1.28 (0.78–2.09)	67/132	50/112	0.90 (0.54–1.48)
Cancer subsite[d]									
Proximal colon	38/264	44/268	0.98 (0.60–1.58)	36/265	44/267	1.16 (0.71–1.88)	35/266	47/266	1.34 (0.82–2.20)
Distal colon	29/264	33/268	0.96 (0.56–1.65)	29/265	33/267	1.03 (0.60–1.77)	36/266	26/266	0.72 (0.42–1.26)
Rectum	26/264	27/268	0.88 (0.49–1.56)	23/265	30/267	1.18 (0.66–2.12)	32/266	21/266	0.66 (0.36–1.19)
Cancer stage[e]									
Stage I, II, III	71/264	82/268	0.98 (0.66–1.44)	69/265	84/267	1.10 (0.74–1.62)	83/266	70/266	0.85 (0.58–1.27)
Stage IV	10/264	19/268	1.61 (0.73–3.57)	11/265	18/267	1.48 (0.67–3.23)	12/266	17/266	1.44 (0.66–3.12)

Abbreviations: BMI = body mass index; CI = confidence interval; CRC = colorectal cancer; CRP = C-reactive protein; IL-6 = interleukin-6; MET = metabolic equivalent = (caloric need per kilogram body weight per hour activity)/(caloric need per kilogram body weight per hour at rest); NSAID = non-steroidal anti-inflammatory drug; RR = relative risk; sTNFR-2 = soluble tumour necrosis factor receptor 2.

[a]Forty two cases diagnosed within 2 years after blood draw were excluded from the analysis. Unconditional logistic regression was used to estimate strata-specific RRs of CRC in men with inflammatory marker levels equal or above the medians among controls relative to men with concentrations below the medians. Multivariate RRs were adjusted for matching factors (age and date of blood collection), BMI, and other variables listed in the footnote for Table 3.

[b]Participants with missing waist circumference ($N = 140$) and waist-to-hip ratio ($N = 141$) were excluded from the stratification analysis by waist circumference and waist-to-hip ratio, respectively.

[c]Regular users were defined as those who used ⩾2 standard (325 mg) tablets of aspirin or ⩾2 tablets of NSAIDs per week.

[d]Forty two cases diagnosed within 2 years after blood draw and 77 cases with missing subsite information were excluded from the analysis. Polytomous logistic regression was used to estimate subsite-specific RRs of CRC after adjusting for matching factors and major risk factors for CRC, including family history, endoscopy screening, pack-years of smoking, alcohol consumption, BMI, physical activity, regular aspirin/NSAID use, and regular use of multivitamins.

[e]Forty two cases diagnosed within 2 years after blood draw and 92 cases with missing stage information were excluded. Polytomous logistic regression was used to estimate stage-specific RRs of CRC after adjusting for BMI and other variables listed in the footnote for Table 3.

and regular aspirin/NSAID use are each related to both inflammatory activity and risk of CRC (Pischon *et al*, 2003a; Chan and Giovannucci, 2010; Khandekar *et al*, 2011). However, prior studies have had varying ability to account for these key factors. For example, previous studies have been unable to adjust for obesity (Il'yasova *et al*, 2005), physical activity (Erlinger *et al*, 2004; Il'yasova *et al*, 2005; Gunter *et al*, 2006) or aspirin/NSAID use (Il'yasova *et al*, 2005; Aleksandrova *et al*, 2010). In the present study, we found our multivariate RRs were substantially attenuated compared with our unadjusted risk estimates, including an association between CRP and CRC, which was no longer significant after multivariate adjustment.

Third, heterogeneity in the study populations may account for differing results. For example, the ATBC Study, which did observe a significant association between CRP and CRC risk, entirely comprised male smokers (Gunter *et al*, 2006). Compared with our cohort, the median and interquartile range of CRP levels among control men in the ATBC study (2.6 mg l^{-1}, 1.4–4.8 mg l^{-1}) were approximately twice as high as those among control men in our present study (1.11 mg l^{-1}, 0.59–2.18 mg l^{-1}), although the effects of different sample sources and assays could not be excluded. In a separate study of a parallel cohort of women, we did observe a significant association between sTNFR-2, but not CRP or IL-6, and risk of CRC (Chan *et al*, 2011). These contrasting results compared with our findings suggest the possibility that the relationship between inflammatory markers and CRC may vary according to patient characteristics, such as smoking status or sex.

Although we did not observe a significant overall association between inflammatory markers and risk of CRC after excluding cases within 2 years of blood collection, we did observe a significant relationship between IL-6 and risk of CRC, which appeared to vary according to BMI. Among men with BMI $<25\,\mathrm{kg\,m^{-2}}$, plasma IL-6 was associated with increased risk of CRC. In contrast, among men with BMI $\geqslant 25\,\mathrm{kg\,m^{-2}}$, IL-6 was not associated with CRC. Recently, chronic inflammation has been implicated as a potential mechanism that mediates the link between obesity and CRC (Khandekar *et al*, 2011). A possible explanation for this observation may be that inflammation-related mechanisms of colorectal carcinogenesis predominate in lean men compared with overweight/obese men for whom other adiposity-related aetiological exposures, such as insulin resistance, hyperinsulinaemia, or dysregulated production of sex steroids (Giovannucci, 2001; Calle and Kaaks, 2004), may be more evident. In support of this explanation, previous studies have demonstrated that two other aetiological pathways for CRC, including the IGF-I axis (for example, IGF-I, IGF-I/IGFBP-3 ratio) and low vitamin D concentration, were also associated with CRC development, primarily among leaner individuals (Ma *et al*, 2004; Wei *et al*, 2005; Wu *et al*, 2007). Alternatively, we hypothesise that compared with overweight men, who demonstrate substantial inflammation in adipose tissues (Khandekar *et al*, 2011), high IL-6 levels in lean men may be more reflective of localised inflammatory events in the colon, which are more relevant to CRC development and can thus confer a greater risk of CRC. In addition, Gunter *et al* (2006) also reported that CRP was more strongly associated with CRC risk among male smokers with a lower BMI in the ATBC cohort. Thus, further studies examining potential mechanisms for the differential associations of inflammatory markers with CRC according to BMI are needed.

Our study has several important strengths. First, our study was prospective with a high follow-up rate. Second, we had detailed information on lifestyle, dietary intake, and medication use, which allowed us to adjust for potential confounding and evaluate possible effect modification. Third, the complete ascertainment of various diseases in this study population enabled us to exclude the potential influence of other illnesses that might be related to both inflammation and CRC, including diabetes mellitus and coronary heart disease.

Our study also has some limitations. First, a single measurement of plasma markers might have caused regression dilution bias (Clarke *et al*, 1999) and precluded us from evaluating associations between the long-term levels of these cytokines and risk of CRC. However, these markers have been shown to be generally stable over time (Pischon *et al*, 2003b; Platz *et al*, 2010). Second, systematic inflammatory markers in plasma might not reflect the tissue-specific inflammatory pathways most relevant to colorectal carcinogenesis. Third, the results mainly from white men may not be generalisable to other populations.

In conclusion, our findings do not appear to support a significant association between plasma inflammatory markers and subsequent risk of CRC among apparently healthy men. However, the possibility that plasma IL-6 may be associated with an increased risk of CRC in lean men requires further investigation.

ACKNOWLEDGEMENTS

We thank the participants and staff of the Health Professionals Follow-up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. We also thank Xuehong Zhang, ScD, for assistance in programming; and Gary Bradwin and Nader Rifai, PhD, for measurements of the inflammatory analytes. This work was supported by US National Institute of Health Grants, P01 CA55075 (to WC Willett), UM1 CA167552 (to WC Willett), P50 CA127003 (to CSF), R01 CA151993 (to SO), and R01 CA137178 and K24 DK 098311 (to ATC). Dr Andrew T Chan is a Damon Runyon Cancer Foundation Clinical Investigator.

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