Research Article

Cancer Epidemiology, Biomarkers & Prevention

Serum Endotoxins and Flagellin and Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort

So Yeon Kong[1], Hao Quang Tran[2], Andrew T. Gewirtz[2], Gail McKeown-Eyssen[3], Veronika Fedirko[4], Isabelle Romieu[1], Anne Tjønneland[5], Anja Olsen[5], Kim Overvad[6], Marie-Christine Boutron-Ruault[7,8,9], Nadia Bastide[7,8,9], Aurélie Affret[7,8,9], Tilman Kühn[10], Rudolf Kaaks[10], Heiner Boeing[11], Krasimira Aleksandrova[11], Antonia Trichopoulou[12,13], Maria Kritikou[12], Effie Vasilopoulou[13], Domenico Palli[14], Vittorio Krogh[15], Amalia Mattiello[16], Rosario Tumino[17], Alessio Naccarati[18], H.B. Bueno-de-Mesquita[19,20,21,22], Petra H. Peeters[23,24], Elisabete Weiderpass[25,26,27,28], J. Ramón Quirós[29], Núria Sala[30], María-José Sánchez[31,32], José María Huerta Castaño[32,33], Aurelio Barricarte[32,34,35], Miren Dorronsoro[36], Mårten Werner[37], Nicholas J. Wareham[38], Kay-Tee Khaw[39], Kathryn E. Bradbury[40], Heinz Freisling[1], Faidra Stavropoulou[1], Pietro Ferrari[1], Marc J. Gunter[21], Amanda J. Cross[21], Elio Riboli[21], W. Robert Bruce[41], and Mazda Jenab[1]

Abstract

Background: Chronic inflammation and oxidative stress are thought to be involved in colorectal cancer development. These processes may contribute to leakage of bacterial products, such as lipopolysaccharide (LPS) and flagellin, across the gut barrier. The objective of this study, nested within a prospective cohort, was to examine associations between circulating LPS and flagellin serum antibody levels and colorectal cancer risk.

Methods: A total of 1,065 incident colorectal cancer cases (colon, $n = 667$; rectal, $n = 398$) were matched (1:1) to control subjects. Serum flagellin- and LPS-specific IgA and IgG levels were quantitated by ELISA. Multivariable conditional logistic regression models were used to calculate ORs and 95% confidence intervals (CI), adjusting for multiple relevant confouding factors.

Results: Overall, elevated anti-LPS and anti-flagellin biomarker levels were not associated with colorectal cancer risk. After testing potential interactions by various factors relevant for colorectal cancer risk and anti-LPS and anti-flagellin, sex was identified as a statistically significant interaction factor ($P_{interaction} < 0.05$ for all the biomarkers). Analyses stratified by sex showed a statistically significant positive colorectal cancer risk association for men (fully-adjusted OR for highest vs. lowest quartile for total anti-LPS + flagellin, 1.66; 95% CI, 1.10–2.51; P_{trend}, 0.049), whereas a borderline statistically significant inverse association was observed for women (fully-adjusted OR, 0.70; 95% CI, 0.47–1.02; P_{trend}, 0.18).

Conclusion: In this prospective study on European populations, we found bacterial exposure levels to be positively associated to colorectal cancer risk among men, whereas in women, a possible inverse association may exist.

Impact: Further studies are warranted to better clarify these preliminary observations. *Cancer Epidemiol Biomarkers Prev; 25(2); 291–301. ©2016 AACR.*

[1]Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC-WHO), Lyon, France. [2]Center for Inflammation, Immunity, and Infection, Department of Biology, Georgia State University, Atlanta, Georgia. [3]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada. [4]Department of Epidemiology, Rollins School of Public Health, Winship Cancer Institute, Emory University, Atlanta, Georgia. [5]Diet, Genes, and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark. [6]Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus C, Denmark. [7]INSERM, CESP Centre for Research in Epidemiology and Population Health, Lifestyle, Genes, and Health: Integrative Trans-Generational Epidemiology, Villejuif, France. [8]University of Paris-South, Villejuif, France. [9]Institute Gustave Roussy, Villejuif, France. [10]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [11]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany. [12]Hellenic Health Foundation, Athens, Greece. [13]Department of Hygiene, Epidemiology, and Medical Statistics, University of Athens Medical School, Athens, Greece. [14]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence, Italy. [15]Epidemiology and Prevention Unit, IRCCS Foundation, National Cancer Institute, Milano, Italy. [16]Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy. [17]Cancer Registry and Histopathology Unit, "Civic – MP Arezzo" Hospital, Ragusa, Italy. [18]Human Genetics Foundation, Torino Molecular and Genetic Epidemiology Unit, Torino, Italy. [19]Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and Environment (RIVM), Bilthoven, the Netherlands. [20]Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, the Netherlands. [21]Department of Epidemiology and Biostatistics, School of Public Health, Imperil College London, London, United Kingdom. [22]Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. [23]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands. [24]MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom. [25]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø,

Introduction

Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of death worldwide (1). It has been postulated that dietary and metabolic factors, such as energy excess and obesity, can cause breakdown of the colonic epithelial barrier function, allowing the interaction of innate immune system with bacterial products, such as lipopolysaccharide (LPS), also known as endotoxin (2). The human gastrointestinal (GI) tract is colonized by a complex community of approximately 10^{14} commensal bacteria, representing approximately 1,000 species (3). Colonic microbiota are being increasingly recognized as important contributors to GI health and likely also to colorectal cancer development (4).

LPS is an integral part of the outer membrane of gram-negative bacterial cell wall and also has a major role in both acute and chronic inflammation (5). A related bacterial product is flagellin, the primary structural component of flagella and a dominant target of humoral immunity in response to infection (6). Emerging evidence suggests that an overabundance of bacterial LPS from the gut microbiota may trigger chronic inflammation and increased production of proinflammatory cytokines and increased reactive oxygen species (2, 7). These proinflammatory cytokines can activate the nuclear factor κβ (NF-κβ) pathway, which has been implicated in cell proliferation and DNA damage leading to carcinogenesis (8). Chronic inflammation has been associated with increased risk of colorectal cancer by several studies (9). Thus, hypothetically, long-term exposure to the localized inflammatory responses resulting from LPS exposure may promote colorectal cancer development.

Direct *in-vivo* measurement of LPS and flagellin levels is challenging, in part because their appearance in blood and organs is sporadic and partly because their presence is quite transient. Hence, a few recent studies have measured levels of immunoglobulins against LPS and flagellin, whose levels can persist for months following exposure to these products, in an attempt to broadly assess systemic exposure to these gut microbial products and probe their potential associations with various disease states (10, 11). In a recent study by Ziegler and colleagues (10), flagellin- and LPS-specific serum immunoglobulin levels (IgM, IgA, and IgG) were markedly increased in patients with short bowel syndrome (SBS) compared with healthy controls. In another study, IgA and IgG antibodies specific for flagellin monomers were shown to be a target of the elevated adaptive immune response associated with Crohn disease, a chronic inflammatory disease of the GI tract (12). Another line of evidence has emerged from a recent animal study that explored the intricate relationship between intestinal barrier function, microbial environment, and inflammation in colorectal cancer by demonstrating that an inflammatory microenvironment promotes colorectal cancer progression in mice (13). The study highlighted that defective intestinal barrier function at tumour sites facilitates invasion of microbial products, triggering inflammation and subsequent tumor growth.

Although the role of microbiota in development of colorectal carcinogenesis has been explored in basic science and animal studies (13, 14), there is currently no direct epidemiologic evidence for the role of endotoxemia and gut barrier dysfunction in colorectal cancer etiology. In the present study, we aimed to examine the association between serum LPS- and flagellin-specific immunoglobulin levels (IgA and IgG) and risk of colorectal cancer development within a nested case–control study in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Materials and Methods

Study population and data collection

We used a case–control design nested within the EPIC cohort, a large prospective cohort study with over 520,000 subjects enrolled from 23 centers in 10 Western European countries (Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom). Details of the design and methods of the EPIC study, including information on dietary assessment methods, blood collection protocols, and follow-up procedures, have been previously described (15). Briefly, individuals who were eligible for the study were selected from the general population of a specific geographical area, town, or province. Exceptions included the French subcohort, which is based on members of the health insurance system or state-school employees, and the Utrecht (Netherlands) subcohort, which is based on women who underwent screening for breast cancer. Between 1992 and 1998, standardized lifestyle and personal history questionnaires, anthropometric data, and blood samples

Norway. [26]Department of Research, Cancer Registry of Norway, Oslo, Norway. [27]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [28]Department of Genetic Epidemiology, Folkhälsan Research Center, Helsinki, Finland. [29]Public Health Directorate, Asturias, Spain. [30]Unit of Nutrition, Environment, and Cancer, Cancer Epidemiology Research Program, and Translational Research Laboratory, Catalan Institute of Oncology (IDIBELL), Barcelona, Spain. [31]Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria ibs, GRANADA, Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain. [32]CIBER de Epidemiología y Salud Pública (CIBERESP), Spain. [33]Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain. [34]Navarra Public Health Institute, Pamplona, Spain. [35]Navarra Institute for Health Research (IdiSNA), Pamplona, Spain. [36]Public Health Direction and CIBERESP-Biodonostia Research Institute, Basque Regional Health Department, San Sebastian, Spain. [37]Institution of Public Health and Medicine, Medicine Umeå University, Umeå, Sweden. [38]MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom. [39]Clinical Gerontology Unit, Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, United Kingdom. [40]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom. [41]Department of Nutritional Sciences, University of Toronto, Toronto, Canada.

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

Current address for F. Stavropoulou: Section of Medical Statistics, Medical University of Vienna, Spitalgasse 23, 1090 Wien, Austria.

Corresponding Authors: Mazda Jenab, International Agency for Research on Cancer (IARC-WHO), 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. Phone: 33-4-72-73-80-82; Fax: 33-4-72-73-83-61; E-mail: jenabm@iarc.fr; and W.R. Bruce, Department of Nutritional Sciences, University of Toronto, 150 College Street, Toronto, Ontario M5S 3E2, Canada. Phone: 1-416-978-5425; Fax: 1-416-978-5882; E-mail: wrbruce@utoronto.ca

doi: 10.1158/1055-9965.EPI-15-0798

were collected from most participants at recruitment. Diet over the previous 12 months was assessed at recruitment by validated country-specific questionnaires designed to ensure high compliance and improved measures of local dietary habits (16).

In each of the study centers, fasting or nonfasting blood samples were drawn from participants who provided a blood sample and stored at 5°C to 10°C, protected from light, and transported to local laboratories for processing and aliquoting as previously described (15, 16). In all countries, except Denmark and Sweden, blood was separated in the local EPIC centeres and stored at the International Agency for Research on Cancer (Lyon, France; −196°C, nitrogen vapor). In Denmark, blood samples were stored locally at −150°C under nitrogen vapor. In Sweden, samples were stored in −80°C freezers.

Follow-up for cancer incidence and vital status

Vital status follow-up (98.4% complete) is collected by record linkage with regional and/or national mortality registries in all countries except Germany and Greece, and the Italian center of Naples, where data are collected actively. Incident cancer cases were determined through record linkage with regional cancer registries (Denmark, other Italian centers, the Netherlands, Norway, Spain, Sweden, and United Kingdom; completed up to June 2003) or via a combination of methods, including linkage with health insurance records, contacts with cancer and pathology registries, and active follow-up through study subjects or their next-of-kin (France, Germany, and Greece; completed up to June 2002). Follow-up began at the date of enrollment and ended at the date of colorectal cancer diagnosis.

Nested case–control study design and selection of study subjects

Case ascertainment and selection. Eligible colorectal cancer cases were first incident, histologically confirmed cases diagnosed within the EPIC study population. Colon cancers were defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, and descending and sigmoid (C18.0–C18.7, according to the 10th Revision of the International Statistical Classification of Diseases, Injury, and Cause of Death), as well as tumors that were overlapping or unspecified (C18.8 and C18.9). Rectal cancers were defined as tumors occurring at the rectosigmoid junction (C19) or rectum (C20). Subjects with anal canal tumors were excluded from the study. Colorectal cancer is defined as a combination of the colon and rectal cancer cases. After exclusions of 23 subjects with missing laboratory measurements of LPS or flagellin and 49 subjects with incomplete matching, a total of 1,065 incident colorectal cancer cases (colon, $n = 667$; rectal, $n = 398$) with available biomarker measurements were included in the study.

Control selection. For each identified cancer case, one control was matched by incidence density sampling by age (within 2.5 years), gender, administrative center, time of the day at blood collection, and fasting status at the time of blood collection (less than 3 hours, 3–6 hours, and more than 6 hours). Women were additionally matched on menopausal status (premenopausal, perimenopausal, postmenopausal, or surgically menopausal). Premenopausal women were further matched on phase of the menstrual cycle at blood collection, and postmenopausal women were matched on current use of hormone replacement therapy. Controls were defined as free of cancer, except nonmelanoma skin cancer, at the time of diagnosis of the case.

Laboratory biomarker measures for serum anti–flagellin- and anti–LPS-specific immunoglobulins

Serum anti–LPS- and anti–flagellin-specific IgA and IgG levels were quantitated by ELISA at Georgia State University as previously described (10, 11). Briefly, microtiter plates (DYNEX) were coated overnight with purified laboratory-made flagellin (100 ng/well) or purified *E.coli* LPS (2 μg/well; from *E. coli* 0128: B12, Sigma; catolog No. 2887) in 9.6 pH bicarbonate buffer. Serum samples from cases and controls diluted at a ratio of 1:200 were applied to wells coated with flagellin or LPS. After incubation and washing, the wells were incubated either with IgG coupled to horseradish peroxidase (GE; catalog No. 375112) or, in the case of IgA-specific antibodies, with peroxidase-labeled IgA (KPL; catolog No. 14-10-01). Quantitation of total immunoglobulins was performed using the colorimetric peroxidase substrate tetramethylbenzidine (TMZ), and optical density (OD) was read at 450 nm and 540 nm (the difference was taken to compensate for optical interference from the plate), with an ELISA plate reader. Data are reported as OD corrected by subtracting background (determined by readings in blank samples) and are normalized to each plate's control sample, which was prepared in bulk, aliquoted, frozen, and thawed daily as used. Only adjusted ODs were used in the analysis. Standardization was performed using preparations of known concentrations of IgA and IgG. Because previously performed assays for these biomarkers in replicates had a very low intraassay coefficient of variation (<5%; ref. 17), our samples were analyzed in singleton to minimize biosample volume requirement, cost, and time. Interassay coefficients of variation were between 3.8% and 6.8%. For all analyses, cases and matched controls were run in the same batch, and the case–control status of the samples was blinded to laboratory technicians.

In the present study, secondary use was made of relevant biomarker measures that had been conducted previously on the same series of subjects (18–20). Briefly, measurements of glycated hemoglobin (HbA1c) were done on erythrocyte hemolysate using the high-performance liquid chromatography method (Bio-Rad Variant II instrument; Bio Lad Laboratories) with intrabatch coefficient of variations of 2.5% (18). High-sensitivity C-reactive protein (hs-CRP) concentrations were measured using a high-sensitivity assay (Beckman-Coulter) on a Synchron LX-20 Pro autoanalyzer (Beckman-Coulter). The interassay coefficients of variation were 6.0% to 6.5% at various concentrations of hs-CRP (19).

Statistical analysis

The distributions of selected characteristics between colon and rectal cases and the matched controls were compared. Normality of each biomarker was checked by visual inspection, and all were deemed to be approximately normal. Each individual biomarker, as well as anti-flagellin (flagellin IgA+flagellin IgG), anti-LPS (LPS IgA+LPS IgG), and anti-flagellin+LPS exposure (flagellin IgA + flagellin IgG + LPS IgA + LPS IgG) levels were categorized into quartiles based on the distribution among the controls with the lowest quartile as the reference category.

Conditional logistic regression was used to estimate the ORs and 95% confidence intervals (CI) of colorectal cancer, and by

anatomical subsite of cancers of colon and rectum in relation to levels of each circulating biomarkers. Risk estimates were computed from both univariate analyses adjusted for the matching factors (matching-adjusted) and multivariable analyses, with additional adjustments for established confounding variables (fully-adjusted), including smoking status (status/duration/intensity of smoking), body mass index (BMI, kg/m^2), waist circumference (cm), education level, total alcohol consumption (g/d), physical activity (sex-specific combined total physical activity index), total energy intake (kcal/day), and total daily intakes of fiber (g/day), fruits and vegetables (g/day), and red/processed meats (g/day; refs. 21–26). For all models, collinearity was assessed, and tests for linear trend were performed using a score variable with values from 1 to 4 included in the model, consistent with the quartile grouping.

We evaluated interactions by several factors relevant for colorectal cancer risk that may be also related to anti–LPS- and anti–flagellin-IgA and -IgG concentrations, total bacterial load, and/or to colonic barrier function (27). Sex and tumor location (colon, rectum) were proposed *a priori* as potential interactions so results are presented stratified by these factors, as well as combined. Other variables (i.e., hs-CRP, waist circumference, BMI, dietary fat, and alcohol intake) were studied for hypothesis generation analyses. Continuous analyses were conducted using a cross-product term of each biomarker and potential interaction term in the model, followed by a likelihood ratio test. Discrete analyses were also undertaken for hs-CRP, waist circumference, BMI, dietary fat, and alcohol intake by including an interaction term formed by the product of the total anti-flagellin+LPS tertile (cutoff points: <5.58, 5.58 to <7.19, ≥7.19) and the sex-specific dichotomized high and low categories of the potential interaction. As with continuous analysis, a likelihood ratio test was used to assess statistical significance.

As a sensitivity analysis, we repeated the main multivariable-adjusted models after excluding cases that occurred in the first 2 years of follow-up and their matched controls to avoid possible reverse causality, as well as after exclusion of countries with lowest (Denmark) and highest (Greece) anti–LPS- and anti-flagellin exposure levels.

Conditional logistic–restricted cubic spline models were used to explore possible deviation from linear relationships between each biomarker and colorectal cancer, with four knots specific at the median of each quartile of biomarker levels (28).

A two-tailed P value of <0.05 was considered to be statistically significant. All statistical analyses were performed with SAS version 9.3 (SAS Institute) statistical software package.

Results

Baseline characteristics of cases and controls

Selected baseline characteristics of the colon and rectal cases and their matched controls are compared in Table 1. Colon and rectal cancer cases were on average 58.8 years and 58.1 years old, respectively. Both colon and rectal cancer cases were more likely to be current smokers, inactive, had higher education, higher total daily energy, and consume less fruit and vegetable than their matched controls. For colon cancer, female cases had lower median concentrations of anti–flagellin-IgA (1.05 vs. 1.17), anti–LPS-IgA (1.56 vs. 1.71), and anti–LPS-IgG (1.36 vs. 1.44) than their matched controls, whereas the concentrations of each of these serologic biomarkers were higher in cases than controls in men (anti–flagellin-IgA: 1.33 vs. 1.30; anti–LPS-IgA: 1.83 vs. 1.68; anti–LPS-IgG: 1.45 vs. 1.37). For rectal cancer, concentrations of each of the serologic biomarkers were higher in cases than controls in women, except anti–LPS-IgA, where cases had lower concentrations than controls (1.38 vs. 1.48). On the other hand, concentrations of all the serologic biomarkers were slightly lower in male rectal cancer cases than controls, except anti–flagellin-IgA, where cases had higher concentrations than controls (1.29 vs. 1.17).

Associations of anti–flagellin- and anti–LPS-IgA and IgG with colorectal cancer

All models found no association between colorectal cancer and biomarkers of either anti-LPS or anti-flagellin (Supplementary Table S1). However, when analyses were stratified by sex, a significant interaction of the colorectal cancer-LPS/flagellin risk association (total anti-flagellin+LPS, $P_{interaction} < 0.05$) was observed. Among men, there was a significant, positive association between colorectal cancer risk and levels of total anti-flagellin+LPS exposure (Table 2) with a fully-adjusted OR of 1.66 (95% CI, 1.10–2.51) comparing the higest versus lowest quartiles, and a significant test for trend (P_{trend} 0.049). In contrast, among women, there were inverse associations with colorectal cancer risk. Anti–flagellin-IgA was negatively associated with risk of colorectal cancer (fully-adjusted OR, 0.65 comparing highest vs. lowest quartiles; 95% CI, 0.44–0.96; $P_{trend} = 0.02$). In addition, there was a trend of significant inverse association between anti–LPS-IgA and risk of colorectal cancer with P_{trend} of 0.02. Unlike among men, the levels of total anti-flagellin+LPS exposure were also negatively related to colorectal cancer risk, though the association did not reach significance (fully-adjusted OR, 0.70 comparing highest vs. lowest quartiles; 95% CI, 0.47–1.02; $P_{trend} = 0.18$).

Associations of anti-LPS and anti-flagellin concentrations with colon and rectal cancer stratified by sex

In stratified analyses by anatomical subsites ($P_{heterogeneity} = 0.64$), colon cancer risk in men continued to be significantly positively associated with total anti-flagellin+LPS concentrations (fully-adjusted OR, 1.80 comparing highest vs. lowest quartiles; 95% CI, 1.04–3.10; $P_{trend} < 0.049$; Table 3), as well as with total anti-LPS (fully-adjusted OR, 1.97; 95% CI, 1.15–3.39; $P_{trend} = 0.01$). However, among women, higher concentrations of several biomarkers remained associated with reduced risk of colon cancer with fully-adjusted ORs of 0.59 (95% CI, 0.37–0.93), 0.57 (95% CI, 0.35–0.91), and 0.62 (95% CI, 0.39–0.98), comparing those with highest quartiles of anti–flagellin-IgA, anti–LPS-IgG, and total anti-LPS to reference, respectively (Table 3).

No significant association was observed between risk of rectal cancer and any of the measures for either men or women (Table 4).

Interactions with inflammation, body size, and dietary fat

The analysis of the interaction between total anti-LPS+flagellin level and inflammation (hs-CRP), body size (waist circumference and BMI), dietary fat intake, and alcohol consumption showed that, among men, the positive association between colorectal cancer risk and total anti-LPS+flagellin level was stronger at higher levels of hs-CRP (OR, 2.35 comparing highest hs-CRP and highest tertile of total anti-LPS+flagellin vs. lowest hs-CRP and lowest tertile of total anti-LPS+flagellin; 95% CI, 1.45–3.81; $P_{interaction} = 0.002$), waist circumference (OR, 1.97; 95% CI,

Table 1. Baseline characteristics of incident colon and rectal cancer cases and matched controls in the EPIC cohort

	Colon cancer		Rectal cancer	
Characteristics	**Cases**	**Controls**	**Cases**	**Controls**
Number	667	667	398	398
Age, years, mean (SD)				
At recruitment	58.8 (7.2)	58.8 (7.3)	58.1 (6.9)	58.0 (6.9)
At blood collection	59.0 (7.3)	59.0 (7.3)	58.1 (6.8)	58.1 (6.8)
Women, *n* (%)	369 (55.3)	369 (55.3)	187 (47.0)	187 (47.0)
BMI, kg/m^2, mean (SD)	26.8 (4.5)	26.3 (3.9)	26.6 (4.1)	26.4 (3.9)
Waist circumference, cm, mean (SD)	90.4 (13.2)	88.0 (12.2)	90.3 (13.1)	89.5 (13.1)
Waist/hip ratio, mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Smoking status/duration/intensity, *n* (%)				
Never-smoker	277 (41.5)	297 (44.5)	155 (38.9)	160 (40.2)
Ex-smokers, duration of smoking < 10 years	40 (6.0)	43 (6.5)	21 (5.3)	30 (7.5)
Ex-smokers, duration of smoking ≥ 10 years	165 (24.7)	164 (24.6)	104 (26.1)	91 (22.9)
Ex-smokers, missing duration of smoking	16 (2.4)	13 (1.9)	4 (1.0)	8 (2.0)
Smokers, <15 cigarettes a day	109 (16.3)	97 (14.5)	79 (19.9)	63 (15.8)
Smokers, ≥15 to <25 cigarettes a day	43 (6.5)	39 (5.9)	24 (6.0)	35 (8.8)
Smokers, ≥25 cigarettes a day	9 (1.4)	6 (0.9)	8 (2.0)	5 (1.3)
Missing smoking status	8 (1.2)	8 (1.2)	3 (0.8)	6 (1.5)
Physical activity, *n* (%)				
Inactive	107 (16.0)	78 (11.7)	59 (14.8)	58 (14.6)
Moderately inactive	202 (30.3)	210 (31.5)	116 (29.2)	103 (25.9)
Moderately active	292 (43.8)	296 (44.4)	176 (44.2)	167 (42.0)
Active	62 (9.3)	77 (11.5)	47 (11.8)	61 (15.3)
Missing/unspecified	4 (0.6)	6 (0.9)	0	9 (2.2)
Education, %				
None/primary school	259 (39.1)	292 (44.0)	150 (38.0)	163 (41.2)
Technical/professional school	158 (23.8)	161 (24.2)	106 (26.8)	109 (27.5)
Secondary school	111 (16.7)	87 (13.1)	54 (13.7)	43 (10.9)
University or higher	117 (17.6)	109 (16.4)	76 (19.2)	76 (19.2)
Missing/unspecified	18 (2.7)	15 (2.3)	9 (2.3)	5 (1.2)
Premenopausal women, *n* (%)	41 (11.1)	42 (11.4)	16 (8.6)	16 (8.6)
Hormone replacement therapy use, *n* (%)	42 (11.5)	40 (10.9)	19 (10.3)	19 (10.3)
Alcohol consumption, g/d, median (IQR)	8.6 (1.3–22.4)	8.4 (1.5–21.1)	11.6 (2.4–31.5)	10.5 (2.2–25.2)
Dietary intakes				
Total energy, kcal/d, median (IQR)	2,066.5 (1,693.2–2,505.2)	2,058.7 (1,729.2–2,453.1)	2,158.6 (1,726.7–2,568.8)	2,093.8 (1,721.3–2,537.8)
Total fats, g/d, median (IQR)	77.4 (60.4–97.5)	77.1 (61.8–97.0)	79.2 (60.2–103.4)	79.6 (63.0–100.5)
Fiber intake, g/d, median (IQR)	22.2 (17.2–27.3)	23.0 (18.4–27.4)	22.0 (18.2–27.7)	22.8 (17.8–28.3)
Fruit and vegetable intake, g/d, median (IQR)	368.9 (244.73–523.9)	417.0 (267.3–566.0)	361.9 (247.8–503.3)	369.9 (251.7–534.4)
Fish and shellfish intake, g/d, median (IQR)	27.0 (14.8–46.7)	29.0 (14.5–50.3)	28.0 (16.0–51.3)	30.0 (14.0–51.5)
Red meat intake, g/d, median (IQR)	48.3 (25.5–77.1)	48.3 (25.8–76.5)	55.2 (33.6–83.1)	54.0 (31.7–81.6)
Processed meat intake, g/d, median (IQR)	25.0 (13.0–40.8)	23.4 (12.5–41.6)	27.3 (13.9–47.5)	26.4 (13.0–46.5)
Fasting status, %				
Yes	25.5	25.5	18.6	18.6
No	48.9	48.9	57.9	57.9
In between	25.6	25.6	23.4	23.4
Blood biomarkers				
Hs-CRP, mg/L, median (IQR)				
Men	2.81 (1.25–5.15)	1.96 (0.89–4.26)	2.13 (1.00–4.31)	2.16 (0.98–4.21)
Women	3.36 (1.28–5.88)	2.59 (1.25–5.11)	2.60 (1.00–4.72)	2.56 (1.09–4.20)
Cholesterol, mmol/L, median (IQR)				
Men	6.07 (5.24–6.86)	6.21 (5.54–6.90)	6.27 (5.57–7.05)	6.22 (5.52–7.00)
Women	6.43 (5.65–7.30)	6.61 (5.77–7.42)	6.55 (5.70–7.30)	6.80 (6.08–7.67)
HDL, mmol/L, median (IQR)				
Men	1.23 (1.04–1.48)	1.29 (1.09–1.60)	1.28 (1.09–1.56)	1.27 (1.07–1.52)
Women	1.51 (1.25–1.78)	1.53 (1.29–1.90)	1.63 (1.33–1.86)	1.61 (1.33–1.86)
LDL, mmol/L, median (IQR)				
Men	3.99 (3.42–4.72)	4.17 (3.57–4.70)	4.16 (3.55–4.86)	4.23 (3.41–4.90)
Women	4.24 (3.56–5.03)	4.30 (3.55–5.06)	4.23 (3.43–4.85)	4.31 (3.70–5.33)
Glycated hemoglobin mg/L, median (IQR)				
Men	5.7 (5.5–6.1)	5.7 (5.5–6.0)	5.7 (5.5–6.0)	5.8 (5.5–6.1)
Women	5.8 (5.5–6.1)	5.7 (5.5–5.9)	5.8 (5.5–6.0)	5.6 (5.5–5.9)
Anti–flagellin-IgA, OD, median (IQR)				
Men	1.33 (0.94–1.79)	1.30 (0.92–1.78)	1.29 (0.85–1.68)	1.17 (0.84–1.67)
Women	1.05 (0.76–1.52)	1.17 (0.80–1.67)	1.01 (0.68–1.47)	0.95 (0.70–1.45)
Anti–flagellin-IgG, OD, median (IQR)				
Men	1.97 (1.42–2.55)	1.99 (1.42–2.51)	1.88 (1.31–2.53)	1.92 (1.43–2.61)
Women	2.03 (1.50–2.63)	2.00 (1.50–2.64)	2.10 (1.48–2.61)	2.02 (1.46–2.58)

(Continued on the following page)

Table 1. Baseline characteristics of incident colon and rectal cancer cases and matched controls in the EPIC cohort (Cont'd)

	Colon cancer		Rectal cancer	
Characteristics	**Cases**	**Controls**	**Cases**	**Controls**
Anti–LPS-IgA, OD, median (IQR)				
Men	1.83 (1.29–2.41)	1.68 (1.28–2.14)	1.62 (1.22–2.26)	1.66 (1.24–2.03)
Women	1.56 (1.19–2.16)	1.71 (1.22–2.24)	1.38 (1.06–1.87)	1.48 (1.04–1.94)
Anti–LPS-IgG, OD, median (IQR)				
Men	1.45 (1.06–1.91)	1.37 (1.08–1.83)	1.28 (1.01–1.85)	1.35 (1.00–1.85)
Women	1.36 (1.00–1.83)	1.44 (1.09–1.93)	1.43 (1.08–1.82)	1.32 (1.01–1.73)

NOTE: Cases and controls were matched on age (within 2.5 years), gender, administrative center, hormone therapy, fasting status, and date of blood collection (within 45 days).

Abbreviations: HDL, high-density lipoprotein; IgA, immunoglobulin A; IgG, immunoglobulin G; IQR, interquartile range; LDL, low-density lipoprotein.

1.24–3.13; $P_{interaction} = 0.01$), BMI (OR, 1.77; 95% CI, 1.13–2.78; $P_{interaction} = 0.03$), and alcohol (OR, 1.71; 95% CI, 1.09–2.69; $P_{interaction} = 0.02$). No interaction was observed in any of these factors among women ($P_{interaction} > 0.05$ for all; Supplementary Table S2).

Sensitivity analysis

After excluding cases that occurred during the first 2 years of follow-up and their matched controls to avoid possible reverse causality, the findings did not change substantially for any of the serologic biomarkers in both colon and rectal cancers for either sex (Supplementary Table S3). Similar results were observed after excluding participants in the countries with lowest (Denmark) and highest (Greece) anti–LPS- and anti-flagellin biomarker exposure levels (data not shown). Spline models showed that the associations between anti-flagellin and anti-LPS biomarkers and risk of colon or rectal cancers were linear (data not shown).

Discussion

In this nested case–control study, we investigated the associations of serologic bacterial markers of anti–LPS- and anti–flagellin-IgA and IgG with colorectal cancer risk. No significant associations were observed with colorectal cancer risk, but subgroup analyses by sex revealed a positive association in men for anti-LPS and anti-flagellin markers combined, whereas in women, the associations were inverse.

One key mechanism whereby microbiota may influence colorectal cancer development is through intestinal barrier dysfunction (29). There is an emerging recognition of the ability of the GI tract to regulate the trafficking of macromolecules between the environment and the host through a barrier mechanism (30). A growing body of evidence supports a link between increased intestinal permeability and several GI disorders such as inflammatory bowel disease (IBD) (31), which is a known risk factor for colorectal cancer. It has been suggested that some dietary/lifestyle exposures (e.g., total fat intake, body weight) and physiologic factors (e.g., inflammation) may exacerbate intestinal permeability, leading to increased exposure of the colonic epithelium to endotoxins and greater leakage of endotoxins into the systemic circulation (32, 33).

The impact of bacteria on the development of colorectal cancer has been mostly studied from the perspective of inflammatory responses. It has become clear that the microbiota has a major influence on immune responses, and chronic inflammation is a well-established risk factor for colorectal cancer (34). LPS has been suggested to be involved in colorectal cancer development through their roles in stimulating the immune system by binding cell-surface Toll-like receptor (TLR)-4, the predominant receptor for LPS, and activating transcription factors, such as NF-κB, resulting in an increased production of proinflammatory cytokines, such as TNFα, IL1, and IL6 (35). Flagellin is recognized by both TLR-5 and the NLRC4 inflammasome, which elicits immune signals by activation of NF-κB and caspase-1, respectively, and hence promotes systemic inflammation by production of multiple inflammatory cytokines (36, 37).

Despite a growing body of evidence from *in vitro* and *in vivo* studies on the role of the microbiome in the development of colorectal cancer, limited epidemiologic studies have thus far been available to show associations between bacterial endotoxin exposure and colorectal adenomas or colorectal cancer. Two recent studies have observed a positive relationship between endotoxin and colorectal adenomas (38, 39) with the strongest associations observed for dysplastic lesions (39). Our results showing a positive association of serum LPS and flagellin biomarkers and colorectal cancer in men are in line with the results of these studies on the role of bacteria exposure in colorectal cancer carcinogenesis. However, these studies did not report sex-stratified findings so do not permit compassion with our findings in women.

We observed in hypothesis-generating analyses that the positive associations between anti-LPS and anti-flagellin levels and risk of colorectal cancer in men were stronger in higher levels of hs-CRP, waist circumference, BMI, and alcohol intake, results which, if replicated, suggest that these factors may play a role in exacerbating the colorectal cancer–promotive effects of LPS and flagellin. Also worthy of examination is the possibility that body size, inflammation, and alcohol intake may influence intestinal permeability and so leads to increased exposure to bacterial products.

Based on the observations from the above-mentioned studies, an inverse association between anti-LPS and anti-flagellin levels and colorectal cancer risk that we observed among women was unexpected. However, other studies have previously demonstrated inverse associations between environmental endotoxin exposures and the risk of lung and other cancers in occupational settings. Protective effects of environmental/occupational endotoxin exposure on lung cancer have been consistently demonstrated in studies of cotton textile due to raw cotton fiber or dust being contaminated with bacterial endotoxin (40–42) and farming industries (43). Differences between men and women have also been observed among cotton plant workers where there was an increased risk of colon and liver cancers in men while women had lower risk of rectal/anal and liver cancers (44). However, these previous studies were based on occupational cohorts with high endotoxin exposures, whereas the endotoxin measures of our study subjects are likely to be derived largely from the colonic bacteria rather than the environment. Therefore, careful interpretation is required when comparing our findings with those of the previous studies looking at specific subject groups.

Table 2. ORs (95% CI) for risk of colorectal cancer by quartile of baseline biomarkers of anti–LPS- and anti–flagellin-IgA and IgG: stratified by sex

Serum immunoglobulins against LPS and flagellin, OD	Continuous (per 1-SD increase) OR (95% CI)	Quartiles[a] Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	P_{trend}[b]
Men						
Anti–Flic-IgA, no. Ca/Co	509/509	94/102	120/128	147/138	148/141	
SD/cutoff point	0.72	≤0.81	>0.81 to ≤ 1.18	>1.18 to ≤ 1.68	>1.68	
Matching-adjusted model[c]	1.03 (0.91–1.18)	1.00	1.01 (0.70–1.46)	1.17 (0.81–1.70)	1.16 (0.80–1.68)	0.35
Fully-adjusted model[d]	1.01 (0.88–1.16)	1.00	1.07 (0.73–1.58)	1.26 (0.85–1.85)	1.16 (0.78–1.71)	0.39
Anti–Flic-IgG, no. Ca/Co	509/509	145/138	121/125	124/124	119/122	
SD/cutoff point	0.78	≤1.46	>1.46 to ≤ 1.98	>1.98 to ≤ 2.58	>2.58	
Matching-adjusted model[c]	0.96 (0.84–1.10)	1.00	0.92 (0.64–1.30)	0.94 (0.65–1.37)	0.92 (0.63–1.33)	0.69
Fully-adjusted model[d]	0.99 (0.85–1.14)	1.00	0.96 (0.66–1.38)	0.96 (0.65–1.43)	0.99 (0.66–1.47)	0.95
Anti–LPS-IgA, no. Ca/Co	509/509	118/115	116/134	120/138	155/122	
SD/cutoff point	0.72	≤1.21	>1.21 to ≤ 1.66	>1.66 to ≤ 2.13	>2.13	
Matching-adjusted model[c]	1.18 (1.03–1.37)	1.00	0.84 (0.58–1.23)	0.87 (0.59–1.28)	1.32 (0.88–1.98)	0.14
Fully-adjusted model[d]	1.17 (1.01–1.36)	1.00	0.82 (0.55–1.21)	0.82 (0.54–1.23)	1.26 (0.82–1.94)	0.27
Anti–LPS-IgG, no. Ca/Co	509/509	135/127	116/130	123/131	135/121	
SD/cutoff point	0.61	≤1.06	>1.06 to ≤ 1.36	>1.36 to ≤ 1.84	>1.84	
Matching-adjusted model[c]	1.08 (0.96–1.23)	1.00	0.83 (0.58–1.20)	0.88 (0.62–1.26)	1.05 (0.72–1.53)	0.72
Fully-adjusted model[d]	1.12 (0.98–1.28)	1.00	0.85 (0.58–1.25)	0.88 (0.61–1.29)	1.13 (0.75–1.68)	0.51
Total anti-Flic, no. Ca/Co	509/509	129/130	113/115	123/133	144/131	
SD/cutoff point	1.23	≤2.47	>2.47 to ≤ 3.19	>3.19 to ≤ 4.05	>4.05	
Matching-adjusted model[c]	0.99 (0.87–1.13)	1.00	1.00 (0.70–1.41)	0.94 (0.66–1.34)	1.12 (0.78–1.62)	0.61
Fully-adjusted model[d]	1.00 (0.87–1.15)	1.00	1.00 (0.70–1.44)	1.00 (0.70–1.45)	1.16 (0.79–1.71)	0.47
Total anti-LPS, no. Ca/Co	509/509	120/115	103/144	129/131	157/119	
SD/cutoff point	1.11	≤2.41	>2.41 to ≤ 3.04	>3.04 to ≤ 3.87	>3.87	
Matching-adjusted model[c]	1.17 (1.02–1.34)	1.00	0.71 (0.50–1.02)	1.01 (0.69–1.47)	1.41 (0.95–2.09)	0.04
Fully-adjusted model[d]	1.18 (1.02–1.37)	1.00	0.71 (0.49–1.04)	0.98 (0.66–1.46)	1.42 (0.94–2.16)	0.04
Total anti-Flic & LPS, no. Ca/Co	509/509	107/127	128/124	123/135	151/123	
SD/cutoff point	2.00	≤5.13	>5.13 to ≤ 6.35	>6.35 to ≤ 7.73	>7.73	
Matching-adjusted model[c]	1.08 (0.95–1.24)	1.00	1.24 (0.87–1.76)	1.12 (0.79–1.59)	1.55 (1.06–2.27)	0.05
Fully-adjusted model[d]	1.09 (0.95–1.26)	1.00	1.31 (0.90–1.90)	1.11 (0.77–1.61)	1.66 (1.10–2.51)	0.05
Women[e]						
Anti–Flic-IgA, no. Ca/Co	556/556	176/165	159/139	122/127	99/125	
Matching-adjusted model[c]	0.87 (0.76–1.00)	1.00	1.03 (0.76–1.41)	0.86 (0.61–1.21)	0.70 (0.49–1.02)	0.04
Fully-adjusted model[d]	0.84 (0.73–0.98)	1.00	0.97 (0.70–1.35)	0.81 (0.57–1.16)	0.65 (0.44–0.96)	0.02
Anti–Flic-IgG, no. Ca/Co	556/556	131/129	139/141	138/142	148/144	
Matching-adjusted model[c]	0.96 (0.85–1.10)	1.00	0.97 (0.69–1.36)	0.96 (0.67–1.36)	1.01 (0.71–1.43)	0.95
Fully-adjusted model[d]	0.98 (0.85–1.12)	1.00	1.01 (0.71–1.44)	1.06 (0.73–1.53)	1.05 (0.73–1.52)	0.74
Anti–LPS-IgA, no. Ca/Co	556/556	165/152	161/133	102/127	128/144	
Matching-adjusted model[c]	0.89 (0.78–1.01)	1.00	1.09 (0.78–1.55)	0.72 (0.50–1.03)	0.78 (0.54–1.11)	0.04
Fully-adjusted model[d]	0.86 (0.75–0.99)	1.00	1.06 (0.74–1.53)	0.67 (0.46–0.98)	0.73 (0.50–1.06)	0.02
Anti–LPS-IgG, no. Ca/Co	556/556	152/141	115/135	158/135	131/145	
Matching-adjusted model[c]	0.95 (0.84–1.08)	1.00	0.78 (0.55–1.10)	1.09 (0.78–1.54)	0.83 (0.58–1.19)	0.64
Fully-adjusted model[d]	0.94 (0.82–1.08)	1.00	0.87 (0.60–1.25)	1.11 (0.78–1.59)	0.83 (0.57–1.21)	0.61
Total anti-Flic, no. Ca/Co	556/556	147/137	136/151	147/132	126/136	
Matching-adjusted model[c]	0.90 (0.79–1.03)	1.00	0.83 (0.59–1.16)	1.02 (0.73–1.42)	0.84 (0.58–1.21)	0.67
Fully-adjusted model[d]	0.89 (0.77–1.03)	1..00	0.83 (0.59–1.18)	1.09 (0.77–1.54)	0.83 (0.56–1.21)	0.72
Total anti-LPS, no. Ca/Co	556/556	162/152	129/122	137/134	128/148	
Matching-adjusted model[c]	0.90 (0.79–1.02)	1.00	0.98 (0.69–1.38)	0.94 (0.67–1.31)	0.77 (0.53–1.10)	0.17
Fully-adjusted model[d]	0.88 (0.76–1.01)	1.00	1.01 (0.71–1.46)	0.91 (0.64–1.30)	0.74 (0.51–1.09)	0.12
Total anti-Flic & LPS, no. Ca/Co	556/556	153/140	139/141	144/132	120/143	
Matching-adjusted model[c]	0.88 (0.77–1.01)	1.00	0.90 (0.65–1.24)	0.99 (0.71–1.39)	0.73 (0.50–1.05)	0.17
Fully-adjusted model[d]	0.86 (0.75–1.00)	1.00	0.89 (0.63–1.25)	1.05 (0.74–1.49)	0.70 (0.47–1.02)	0.18

Abbreviations: Ca/Co, case/control; Flic, flagellin; Total anti-Flic, anti–flagellin-IgA + anti–flagellin-IgG; Total anti-LPS, anti–LPS-IgA + anti–LPS-IgG; Total anti-Flic & LPS, anti–flagellin-IgA + anti–flagellin-IgG + anti–LPS-IgA + anti–LPS-IgG.

[a]Quartile cutoff points were based on the distribution of controls, expressed as OD readings.

[b]P_{trend} test was based on median values of each quartile.

[c]Matching-adjusted model based on logistic regression conditioned on matching factors (age, gender, administrative center, and date of blood collection).

[d]Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, BMI, weight circumference, physical activity, education, and total daily dietary energy consumption, fiber intake, fruits and vegetable intakes, and meat and processed meat consumption).

[e]Quartile cutoff points are same as those in men.

Two mechanisms may be involved in the differences between men and women that we observed in the associations between endotoxin and risk of colorectal cancer. First, complex interactions between the innate and adaptive immune systems are important underlying mechanisms of associations between endotoxin and carcinogenesis (45). The differences between men and women are observed and could therefore result from well-established sex-based differences in the immune systems that result in women having a more vigorous immune response, both cellular and humoral, than men (46–48).

Table 3. ORs (95% CI) for risk of colon cancer by quartile of baseline biomarkers of anti–LPS- and anti–flagellin-IgA and IgG: stratified by sex

Serum immunoglobulins against LPS and flagellin, OD	Continuous (per 1-SD increase) OR (95% CI)	Quartiles[a] Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	P_{trend}[b]
Men						
Anti–Flic-IgA, no. Ca/Co	298/298	55/61	79/72	79/84	85/81	
SD/cutoff point	0.74	≤0.85	>0.85 to ≤ 1.23	>1.23 to ≤ 1.72	>1.72	
Matching-adjusted model[c]	1.01 (0.85–1.20)	1.00	1.20 (0.75–1.93)	1.03 (0.64–1.67)	1.14 (0.71–1.83)	0.73
Fully-adjusted model[d]	0.99 (0.83–1.19)	1.00	1.29 (0.78–2.13)	1.13 (0.67–1.88)	1.09 (0.66–1.80)	0.91
Anti–Flic-IgG, no. Ca/Co	298/298	77/83	76/67	76/79	69/69	
SD/cutoff point	0.79	≤1.47	>1.47 to ≤ 2.00	>2.00 to ≤ 2.58	>2.58	
Matching-adjusted model[c]	1.00 (0.84–1.20)	1.00	1.25 (0.77–2.02)	1.06 (0.65–1.74)	1.10 (0.66–1.82)	0.90
Fully-adjusted model[d]	1.04 (0.86–1.27)	1.00	1.37 (0.82–2.29)	1.23 (0.72–2.09)	1.25 (0.72–2.17)	0.54
Anti–LPS-IgA, no. Ca/Co	298/298	69/69	67/83	71/77	91/69	
SD/cutoff point	0.72	≤1.23	>1.23 to ≤ 1.70	>1.70 to ≤ 2.20	>2.20	
Matching-adjusted model[c]	1.19 (0.99–1.43)	1.00	0.81 (0.51–1.31)	0.95 (0.58–1.55)	1.46 (0.87–2.45)	0.12
Fully-adjusted model[d]	1.18 (0.97–1.44)	1.00	0.85 (0.51–1.41)	0.93 (0.54–1.60)	1.44 (0.83–2.51)	0.18
Anti–LPS-IgG, no. Ca/Co	298/298	82/76	61/81	74/77	81/64	
SD/cutoff point	0.60	≤1.08	>1.08 to ≤ 1.41	>1.41 to ≤ 1.86	>1.86	
Matching-adjusted model[c]	1.14 (0.97–1.35)	1.00	0.69 (0.43–1.11)	0.88 (0.55–1.40)	1.19 (0.73–1.94)	0.33
Fully-adjusted model[d]	1.20 (1.00–1.44)	1.00	0.77 (0.46–1.28)	0.90 (0.54–1.49)	1.34 (0.79–2.28)	0.21
Total anti-Flic, no. Ca/Co	298/298	69/76	75/70	71/79	83/73	
SD/cutoff point	1.26	≤2.50	>2.50 to ≤ 3.26	>3.26 to ≤ 4.11	>4.11	
Matching-adjusted model[c]	1.01 (0.85–1.20)	1.00	1.21 (0.75–1.95)	1.01 (0.63–1.62)	1.31 (0.80–2.15)	0.45
Fully-adjusted model[d]	1.02 (0.84–1.22)	1.00	1.21 (0.73–2.01)	1.11 (0.67–1.85)	1.30 (0.76–2.23)	0.43
Total anti-LPS, no. Ca/Co	298/298	63/73	68/83	72/74	95/68	
SD/cutoff point	1.10	≤2.47	>2.47 to ≤ 3.10	>3.10 to ≤ 3.93	>3.93	
Matching-adjusted model[c]	1.22 (1.02–1.46)	1.00	0.97 (0.62–1.51)	1.18 (0.74–1.90)	1.83 (1.11–3.02)	0.01
Fully-adjusted model[d]	1.25 (1.03–1.53)	1.00	1.11 (0.69–1.78)	1.26 (0.75–2.10)	1.97 (1.15–3.39)	0.01
Total anti-Flic & LPS, no. Ca/Co	298/298	61/76	78/80	71/70	88/72	
SD/cutoff point	2.00	≤5.28	>5.28 to ≤ 6.46	>6.46 to ≤ 7.91	>7.91	
Matching-adjusted model[c]	1.11 (0.94–1.33)	1.00	1.22 (0.78–1.91)	1.33 (0.83–2.13)	1.65 (1.00–2.72)	0.05
Fully-adjusted model[d]	1.14 (0.94–1.37)	1.00	1.42 (0.88–2.31)	1.42 (0.86–2.37)	1.80 (1.04–3.10)	0.049
Women[e]						
Anti–Flic-IgA, no. Ca/Co	369/369	119/107	103/94	86/83	61/85	
Matching-adjusted model[c]	0.83 (0.70–0.97)	1.00	0.96 (0.66–1.40)	0.89 (0.59–1.32)	0.63 (0.40–0.97)	0.05
Fully-adjusted model[d]	0.80 (0.36–0.96)	1.00	0.94 (0.63–1.39)	0.84 (0.55–1.29)	0.59 (0.37–0.93)	0.03
Anti–Flic-IgG, no. Ca/Co	369/369	89/84	94/100	87/88	99/97	
Matching-adjusted model[c]	0.94 (0.80–1.10)	1.00	0.89 (0.60–1.33)	0.93 (0.61–1.44)	0.96 (0.62–1.48)	0.94
Fully-adjusted model[d]	0.95 (0.80–1.13)	1.00	0.94 (0.62–1.44)	1.07 (0.68–1.69)	1.01 (0.64–1.60)	0.84
Anti–LPS-IgA, no. Ca/Co	369/369	104/98	112/84	65/90	88/97	
Matching-adjusted model[c]	0.90 (0.77–1.05)	1.00	1.27 (0.83–1.94)	0.68 (0.43–1.05)	0.84 (0.55–1.29)	0.10
Fully-adjusted model[d]	0.89 (0.75–1.04)	1.0	1.26 (0.80–1.99)	0.66 (0.42–1.06)	0.81 (0.51–1.28)	0.08
Anti–LPS-IgG, no. Ca/Co	369/369	117/91	78/86	91/90	83/102	
Matching-adjusted model[c]	0.85 (0.72–0.99)	1.00	0.68 (0.44–1.05)	0.76 (0.51–1.15)	0.58 (0.37–0.90)	0.03
Fully-adjusted model[d]	0.84 (0.71–0.99)	1.00	0.74 (0.47–1.16)	0.74 (0.48–1.14)	0.57 (0.35–0.91)	0.02
Total anti-Flic, no. Ca/Co	369/369	96/91	104/97	91/88	78/93	
Matching-adjusted model[c]	0.85 (0.72–1.01)	1.00	1.00 (0.67–1.50)	0.97 (0.64–1.47)	0.76 (0.49–1.19)	0.26
Fully-adjusted model[d]	0.85 (0.71–1.01)	1.00	1.08 (0.71–1.66)	1.10 (0.71–1.72)	0.75 (0.46–1.21)	0.31
Total anti-LPS, no. Ca/Co	369/369	116/94	81/85	89/92	83/98	
Matching-adjusted model[c]	0.85 (0.73–0.99)	1.00	0.76 (0.50–1.14)	0.77 (0.52–1.15)	0.64 (0.42–0.99)	0.06
Fully-adjusted model[d]	0.84 (0.71–0.99)	1.00	0.74 (0.48–1.15)	0.75 (0.49–1.15)	0.62 (0.39–0.98)	0.049
Total anti-Flic & LPS, no. Ca/Co	369/369	109/91	93/87	87/97	80/94	
Matching-adjusted model[c]	0.83 (0.71–0.98)	1.00	0.88 (0.59–1.33)	0.73 (0.48–1.11)	0.68 (0.44–1.05)	0.05
Fully-adjusted model[d]	0.82 (0.69–0.97)	1.00	0.90 (0.58–1.39)	0.80 (0.52–1.24)	0.66 (0.42–1.05)	0.07

Abbreviations: Ca/Co, case/control; Flic, flagellin; Total anti-Flic, anti–flagellin-IgA + anti–flagellin-IgG; Total anti-LPS, anti–LPS-IgA + anti–LPS-IgG; Total anti-Flic & LPS, anti–flagellin-IgA + anti–flagellin-IgG + anti–LPS-IgA + anti–LPS-IgG.

[a]Quartile cutoff points were based on the distribution of controls, expressed as OD readings.

[b]P_{trend} test was based on median values of each quartile.

[c]Matching-adjusted model based on logistic regression conditioned on matching factors (age, gender, administrative center, and date of blood collection).

[d]Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, BMI, weight circumference, physical activity, education, and total daily dietary energy consumption, fiber intake, fruits and vegetable intakes, and meat and processed meat consumption).

[e]Quartile cutoff points are same as those in men.

Second, it is possible that the composition of microbiota could differ in men and women as sex differences have been observed in the composition of skin microbiota (49). It is therefore possible that different organisms might have different associations with colon carcinogenesis and so account for the differences between men and women we observed. Such possibilities have yet to be studied in detail.

Lastly, it is possible that our gender-specific observations are due to chance, despite the relatively large size of the present study. Therefore, replication of these findings and deeper exploration of

Table 4. ORs (95% CI) for risk of rectal cancer by quartile of baseline biomarkers of anti–LPS- and anti–flagellin-IgA and IgG: stratified by sex

Serum immunoglobulins against LPS and flagellin, OD	Continuous (per 1-SD increase) OR (95% CI)	Quartiles[a] Q1	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	P_{trend}[b]
Men						
Anti–Flic-IgA, no. Ca/Co	211/211	38/42	39/49	72/60	62/60	
SD/cutoff point	0.69	≤0.75	>0.75 to ≤1.06	>1.06 to ≤1.59	> 1.59	
Matching-adjusted model[c]	1.07 (0.88–1.31)	1.00	0.87 (0.48–1.60)	1.32 (0.76–2.28)	1.17 (0.64–2.14)	0.34
Fully-adjusted model[d]	1.08 (0.87–1.33)	1.00	0.98 (0.50–1.92)	1.34 (0.75–2.41)	1.30 (0.68–2.49)	0.28
Anti–Flic-IgG, no. Ca/Co	211/211	65/54	48/58	52/46	46/53	
SD/cutoff point	0.76	≤1.45	>1.45 to ≤1.95	>1.95 to ≤2.60	>2.60	
Matching-adjusted model[c]	0.90 (0.73–1.11)	1.00	0.70 (0.42–1.17)	0.95 (0.54–1.67)	0.72 (0.40–1.29)	0.37
Fully-adjusted model[d]	0.95 (0.76–1.19)	1.00	0.69 (0.39–1.23)	0.94 (0.51–1.71)	0.78 (0.41–1.48)	0.57
Anti–LPS-IgA, no. Ca/Co	211/211	40/44	62/52	45/61	64/54	
SD/cut off point	0.71	≤1.14	>1.14 to ≤1.57	>1.57 to ≤2.02	>2.02	
Matching-adjusted model[c]	1.17 (0.94–1.46)	1.00	1.30 (0.71–2.37)	0.80 (0.41–1.58)	1.33 (0.68–2.62)	0.67
Fully-adjusted model[d]	1.19 (0.93–1.53)	1.00	1.43 (0.75–2.73)	0.79 (0.38–1.66)	1.40 (0.67–2.94)	0.68
Anti–LPS-IgG, no. Ca/Co	211/211	53/53	61/49	44/54	53/55	
SD/cutoff point	0.61	≤1.01	>1.01 to ≤1.33	>1.33 to ≤1.78	>1.78	
Matching-adjusted model[c]	1.01 (0.84–1.23)	1.00	1.26 (0.73–2.15)	0.81 (0.47–1.40)	0.92 (0.52–1.63)	0.51
Fully-adjusted model[d]	1.04 (0.85–1.28)	1.00	1.20 (0.67–2.14)	0.78 (0.43–1.39)	0.98 (0.53–1.80)	0.63
Total anti-Flic, no. Ca/Co	211/211	59/56	44/44	52/54	56/57	
SD/cutoff point	1.17	≤2.40	>2.40 to ≤3.10	>3.10 to ≤3.99	>3.99	
Matching-adjusted model[c]	0.98 (0.80–1.19)	1.00	0.95 (0.55–1.64)	0.91 (0.54–1.55)	0.93 (0.54–1.59)	0.75
Fully-adjusted model[d]	1.01 (0.82–1.25)	1.00	0.95 (0.53–1.68)	0.92 (0.52–1.61)	1.01 (0.56–1.81)	0.99
Total anti-LPS, no. Ca/Co	211/211	53/42	36/61	59/58	63/50	
SD/cutoff point	1.12	≤2.28	>2.28 to ≤2.91	>2.91 to ≤3.75	>3.75	
Matching-adjusted model[c]	1.11 (0.89–1.37)	1.00	0.44 (0.23–0.82)	0.84 (0.46–1.54)	1.06 (0.56–2.01)	0.42
Fully-adjusted model[d]	1.13 (0.90–1.43)	1.00	0.37 (0.19–0.72)	0.77 (0.40–1.50)	1.16 (0.58–2.32)	0.36
Total anti-Flic & LPS, no. Ca/Co	211/211	43/52	50/46	62/59	56/54	
SD/cutoff point	1.98	≤4.95	>4.95 to ≤6.11	>6.11 to ≤7.55	>7.55	
Matching-adjusted model[c]	1.04 (0.85–1.28)	1.00	1.34 (0.75–2.41)	1.29 (0.75–2.21)	1.30 (0.71–2.39)	0.44
Fully-adjusted model[d]	1.08 (0.86–1.35)	1.00	1.27 (0.68–2.39)	1.24 (0.69–2.21)	1.49 (0.77–2.90)	0.29
Women[e]						
Anti–Flic-IgA, no. Ca/Co	187/187	61/58	43/50	41/40	42/39	
Matching-adjusted model[c]	0.99 (0.77–1.28)	1.00	0.84 (0.49–1.43)	0.98 (0.52–1.87)	1.04 (0.54–2.00)	0.83
Fully-adjusted model[d]	0.96 (0.73–1.26)	1.00	0.72 (0.41–1.26)	0.82 (0.41–1.64)	0.95 (0.47–1.92)	0.92
Anti–Flic-IgG, no. Ca/Co	187/187	43/46	46/41	49/54	49/46	
Matching-adjusted model[c]	1.01 (0.82–1.26)	1.00	1.18 (0.65–2.16)	0.98 (0.54–1.79)	1.13 (0.62–2.07)	0.84
Fully-adjusted model[d]	1.01 (0.80–1.28)	1.00	1.17 (0.62–2.20)	1.06 (0.55–2.02)	1.15 (0.60–2.21)	0.77
Anti–LPS-IgA, no. Ca/Co	187/187	53/56	58/47	37/40	39/44	
Matching-adjusted model[c]	0.86 (0.68–1.10)	1.00	1.32 (0.73–2.40)	0.98 (0.53–1.81)	0.92 (0.46–1.83)	0.58
Fully-adjusted model[d]	0.82 (0.63–1.07)	1.00	1.34 (0.71–2.52)	0.89 (0.47–1.71)	0.84 (0.40–1.74)	0.39
Anti–LPS-IgG, no. Ca/Co	187/187	39/47	39/50	61/46	48/44	
Matching-adjusted model[c]	1.21 (0.96–1.53)	1.00	0.94 (0.52–1.72)	1.94 (1.01–3.73)	1.60 (0.83–3.09)	0.10
Fully-adjusted model[d]	1.22 (0.96–1.56)	1.00	1.11 (0.59–2.10)	2.21 (1.11–4.40)	1.74 (0.87–3.50)	0.07
Total anti-Flic, no. Ca/Co	187/187	49/44	38/55	57/46	43/42	
Matching-adjusted model[c]	1.01 (0.80–1.27)	1.00	0.64 (0.36–1.14)	1.10 (0.62–1.96)	0.93 (0.50–1.75)	0.69
Fully-adjusted model[d]	0.99 (0.77–1.27)	1.00	0.61 (0.33–1.11)	1.10 (0.59–2.03)	0.92 (0.47–1.80)	0.73
Total anti-LPS, no. Ca/Co	187/187	49/58	43/38	49/41	46/50	
Matching-adjusted model[c]	1.03 (0.81–1.32)	1.00	1.38 (0.75–2.54)	1.48 (0.80–2.75)	1.15 (0.61–2.17)	0.64
Fully-adjusted model[d]	1.00 (0.77–1.31)	1.00	1.49 (0.78–2.85)	1.44 (0.77–2.72)	1.13 (0.58–2.23)	0.73
Total anti-Flic & LPS, no. Ca/Co	187/187	49/48	45/53	50/42	43/44	
Matching-adjusted model[c]	1.02 (0.80–1.31)	1.00	0.84 (0.48–1.48)	1.16 (0.64–2.08)	0.95 (0.50–1.79)	0.80
Fully-adjusted model[d]	1.00 (0.76–1.30)	1.00	0.80 (0.44–1.46)	1.17 (0.63–2.19)	0.91 (0.46–1.78)	0.85

Abbreviations: Ca/Co, case/control; Flic, flagellin; Total anti-Flic, anti–flagellin-IgA + anti–flagellin-IgG; Total anti-LPS, anti–LPS-IgA + anti–LPS-IgG; Total anti-Flic & LPS, anti–flagellin-IgA + anti–flagellin-IgG + anti–LPS-IgA + anti–LPS-IgG.

[a]Quartile cutoff points were based on the distribution of controls, expressed as OD readings.

[b]P_{trend} test was based on median values of each quartile.

[c]Matching-adjusted model based on logistic regression conditioned on matching factors (age, gender, administrative center, and date of blood collection).

[d]Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, BMI, weight circumference, physical activity, education, and total daily dietary energy consumption, fiber intake, fruits and vegetable intakes, and meat and processed meat consumption).

[e]Quartile cutoff points are same as those in men.

the sex-specific bacterial exposure and colorectal cancer hypothesis is required.

The present study has several strengths. Our study is the largest prospective cohort so far to investigate bacterial exposures and colorectal cancer risk. Therefore, we had a large enough sample size to be able to stratify by anatomical subsites of colorectal cancer and by gender. To our knowledge, no previous studies on bacterial exposure and colorectal cancer risk have had a sufficiently large enough sample size to conduct stratified analyses.

We also have several limitations in the study. First, because the gut is colonized by complex bacterial communities, elevated anti-LPS or anti-flagellin levels alone may not be sufficient to promote inflammation and tumor progression (38). Another limitation is that we measured the anti-LPS and anti-flagellin concentrations in serum, not in the colonic mucosa, which could be more relevant for colorectal carcinoma formation. Indeed, the assay we applied measures serum immunoreactivity to common bacterial flagellin monomers, which have highly conserved regions common to many flagellins in the microbiota. Although differences in such flagellin immunoreactivity have been thought to reflect differences in gut permeability, they may also arise from differences in microbiota composition and/or gene expression. Thus, better clarification of the source and biologic properties of these compounds is a task for future research. Moreover, biosamples were available only from the time of recruitment into the cohort, and thus we only had a single-blood measure taken at one point in time.

In summary, we found no overall association between bacterial exposure levels, measured by anti–LPS- and anti-flagellin-IgA and IgG, and risk of colorectal cancer. However, in subgroup analysis by sex, we found some biomarker levels to be positively associated with colorectal cancer risk among men, whereas they were inversely associated with colorectal cancer risk among women. Further studies are warranted to elucidate the underlying mechanisms of bacterial exposure and colorectal cancer by sex as well as the sex-specific role of inflammation and immune response on colorectal cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Authors' Contributions

Conception and design: G. McKeown-Eyssen, A. Tjønneland, K. Overvad, V. Krogh, R. Tumino, H.B. Bueno-de-Mesquita, P.H. Peeters, E. Weiderpass, M.-J. Sánchez, A. Barricarte, M. Dorronsoro, K.-T. Khaw, H. Freisling, W.R. Bruce, M. Jenab

Development of methodology: G. McKeown-Eyssen, R. Tumino, H.B. Bueno-de-Mesquita, E. Weiderpass, A. Barricarte, M. Dorronsoro, M. Jenab

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.Q. Tran, A.T. Gewirtz, K. Overvad, M.-C. Boutron-Ruault, T. Kühn, R. Kaaks, H. Boeing, A. Trichopoulou, E. Vasilopoulou, D. Palli, V. Krogh, A. Mattiello, R. Tumino, A. Naccarati, H.B. Bueno-de-Mesquita, P.H. Peeters, E. Weiderpass, J.R. Quirós, N. Sala, M.-J. Sánchez, A. Barricarte, M. Dorronsoro, M. Werner, N.J. Wareham, K.-T. Khaw, K.E. Bradbury, M. Jenab

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.Y. Kong, H.Q. Tran, A.T. Gewirtz, G. McKeown-Eyssen, V. Fedirko, A. Tjønneland, D. Palli, E. Weiderpass, M. Werner, N.J. Wareham, H. Freisling, P. Ferrari, M.J. Gunter, M. Jenab

Writing, review, and/or revision of the manuscript: S.Y. Kong, G. McKeown-Eyssen, V. Fedirko, I. Romieu, A. Tjønneland, A. Olsen, K. Overvad, M.-C. Boutron-Ruault, N. Bastide, A. Affret, T. Kühn, R. Kaaks, H. Boeing, K. Aleksandrova, A. Trichopoulou, M. Kritikou, E. Vasilopoulou, D. Palli, V. Krogh, A. Mattiello, A. Naccarati, H.B. Bueno-de-Mesquita, P.H. Peeters, E. Weiderpass, J.R. Quirós, N. Sala, M.-J. Sánchez, J.M. Huerta Castaño, A. Barricarte, M. Dorronsoro, M. Werner, N.J. Wareham, K.-T. Khaw, K.E. Bradbury, H. Freisling, F. Stavropoulou, P. Ferrari, M.J. Gunter, A.J. Cross, E. Riboli, W.R. Bruce, M. Jenab

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.Y. Kong, T. Kühn, H. Boeing, M. Kritikou, R. Tumino, E. Weiderpass, J.R. Quirós, K.-T. Khaw, K.E. Bradbury, M. Jenab

Study supervision: M. Kritikou, R. Tumino, H.B. Bueno-de-Mesquita, P.H. Peeters, E. Weiderpass, M. Dorronsoro, M. Jenab

Other (co-author on the grant which supported the research): G. McKeown-Eyssen

Grant Support

This work was funded by Wereld Kanker Onderzoek Fonds as part of the World Cancer Research Fund (WCRF) International Regular Grant Programme (grant number 2010–251; pricipal investigator: M. Jenab). The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM, France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare, and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (the Netherlands); Nordic Centre of Excellence programme on Food, Nutrition, and Health (Norway); Health Research Fund (FIS), PI13/00061 to Granada, Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236) and Navarra, ISCIII RETIC (RD06/0020; Spain); Swedish Cancer Society, Swedish Scientific Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 to EPIC-Oxford; K.E. Bradbury and K.-T. Khaw), Medical Research Council (1000143 to EPIC-Norfolk, United Kingdom).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 10, 2015; revised November 16, 2015; accepted December 11, 2015; published OnlineFirst January 11, 2016.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
2. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. Curr Opin Clin Nutr Metab Care 2007;10:729–34.
3. Ashida H, Ogawa M, Kim M, Mimuro H, Sasakawa C. Bacteria and host interactions in the gut epithelial barrier. Nat Chem Biol 2012;8:36–45.
4. Ahn J, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, et al. Human gut microbiome and risk for colorectal cancer. J Natl Cancer Inst 2013;105:1907–11.
5. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. Endotoxemia is associated with an increased risk of incident diabetes. Diabetes Care 2011;34:392–7.
6. Sanders CJ, Yu Y, Moore DA 3rd, Williams IR, Gewirtz AT. Humoral immune response to flagellin requires T cells and activation of innate immunity. J Immunol 2006;177:2810–8.
7. Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MC, et al. Energy intake is associated with endotoxemia in apparently healthy men. Am J Clin Nutr 2008;87:1219–23.
8. Atreya R, Neurath MF. Signaling molecules: the pathogenic role of the IL-6/STAT-3 trans signaling pathway in intestinal inflammation and in colonic cancer. Curr Drug Targets 2008;9:369–74.
9. Nimptsch K, Aleksandrova K, Boeing H, Janke J, Lee YA, Jenab M, et al. Association of CRP genetic variants with blood concentrations of C-reactive protein and colorectal cancer risk. Int J Cancer 2015;136:1181–92.

10. Ziegler TR, Luo M, Estivariz CF, Moore DA 3rd, Sitaraman SV, Hao L, et al. Detectable serum flagellin and lipopolysaccharide and upregulated anti-flagellin and lipopolysaccharide immunoglobulins in human short bowel syndrome. Am J Physiol Regul Integr Comp Physiol 2008;294:R402–10.
11. Sitaraman SV, Klapproth JM, Moore DA 3rd, Landers C, Targan S, Williams IR, et al. Elevated flagellin-specific immunoglobulins in Crohn's disease. Am J Physiol Gastrointest Liver Physiol 2005;288:G403–6.
12. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, et al. Bacterial flagellin is a dominant antigen in Crohn disease. J Clin Invest 2004;113:1296–306.
13. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012;491:254–8.
14. Hope ME, Hold GL, Kain R, El-Omar EM. Sporadic colorectal cancer–role of the commensal microbiota. FEMS Microbiol Lett 2005;244:1–7.
15. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997; 26:S6–14.
16. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
17. Gewirtz AT, Vijay-Kumar M, Brant SR, Duerr RH, Nicolae DL, Cho JH. Dominant-negative TLR5 polymorphism reduces adaptive immune response to flagellin and negatively associates with Crohn's disease. Am J Physiol Gastrointest Liver Physiol 2006;290:G1157–63.
18. Rinaldi S, Rohrmann S, Jenab M, Biessy C, Sieri S, Palli D, et al. Glycosylated hemoglobin and risk of colorectal cancer in men and women, the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev 2008;17:3108–15.
19. Aleksandrova K, Jenab M, Boeing H, Jansen E, Bueno-de-Mesquita HB, Rinaldi S, et al. Circulating C-reactive protein concentrations and risks of colon and rectal cancer: a nested case-control study within the European Prospective Investigation into Cancer and Nutrition. Am J Epidemiol 2010; 172:407–18.
20. Aleksandrova K, Boeing H, Jenab M, BasBueno-de-Mesquita H, Jansen E, van Duijnhoven FJ, et al. Metabolic syndrome and risks of colon and rectal cancer: the European prospective investigation into cancer and nutrition study. Cancer Prev Res 2011;4:1873–83.
21. Murphy N, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, et al. Dietary fibre intake and risks of cancers of the colon and rectum in the European prospective investigation into cancer and nutrition (EPIC). PLoS One 2012;7:e39361.
22. van Duijnhoven FJ, Bueno-De-Mesquita HB, Ferrari P, Jenab M, Boshuizen HC, Ros MM, et al. Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr 2009;89:1441–52.
23. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 2005;97:906–16.
24. Leufkens AM, Van Duijnhoven FJ, Siersema PD, Boshuizen HC, Vrieling A, Agudo A, et al. Cigarette smoking and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition Study. Clin Gastroenterol Hepatol 2011;9:137–44.
25. Steins Bisschop CN, van Gils CH, Emaus MJ, Bueno-de-Mesquita HB, Monninkhof EM, Boeing H, et al. Weight change later in life and colon and rectal cancer risk in participants in the EPIC-PANACEA study1,3. Am J Clin Nutr 2014;99:139–47.
26. Ferrari P, Jenab M, Norat T, Moskal A, Slimani N, Olsen A, et al. Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer 2007;121:2065–72.
27. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–81.
28. Li R, Hertzmark E, Louie M, Chen L, Spiegelman D. The SAS LGTPHCURV9 Macro. 2010.
29. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013; 13:800–12.
30. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev 2011;91:151–75.
31. Shen L, Su L, Turner JR. Mechanisms and functional implications of intestinal barrier defects. Dig Dis 2009;27:443–9.
32. Teixeira TF, Collado MC, Ferreira CL, Bressan J, Peluzio Mdo C. Potential mechanisms for the emerging link between obesity and increased intestinal permeability. Nutr Res 2012;32:637–47.
33. Burcelin R, Serino M, Chabo C, Blasco-Baque V, Amar J. Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. Acta Diabetol 2011;48:257–73.
34. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol 2014;12:661–72.
35. Killeen SD, Wang JH, Andrews EJ, Redmond HP. Bacterial endotoxin enhances colorectal cancer cell adhesion and invasion through TLR-4 and NF-kappaB-dependent activation of the urokinase plasminogen activator system. Br J Cancer 2009;100:1589–602.
36. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001;410:1099–103.
37. Steiner TS. How flagellin and toll-like receptor 5 contribute to enteric infection. Infect Immun 2007;75:545–52.
38. Kang M, Edmundson P, Araujo-Perez F, McCoy AN, Galanko J, Keku TO. Association of plasma endotoxin, inflammatory cytokines and risk of colorectal adenomas. BMC Cancer 2013;13:91.
39. Lee KK, Yum KS. Association of endotoxins and colon polyp: a case-control study. J Korean Med Sci 2012;27:1062–5.
40. Wernli KJ, Ray RM, Gao DL, Thomas DB, Checkoway H. Cancer among women textile workers in Shanghai, China: overall incidence patterns, 1989–1998. Am J Ind Med 2003;44:595–9.
41. Hodgson JT, Jones RD. Mortality of workers in the British cotton industry in 1968–1984. Scand J Work Environ Health 1990;16:113–20.
42. Astrakianakis G, Seixas NS, Ray R, Camp JE, Gao DL, Feng Z, et al. Lung cancer risk among female textile workers exposed to endotoxin. J Natl Cancer Inst 2007;99:357–64.
43. Blair A, Sandler DP, Tarone R, Lubin J, Thomas K, Hoppin JA, et al. Mortality among participants in the agricultural health study. Ann Epidemiol 2005;15:279–85.
44. Szeszenia-Dabrowska N, Wilczynska U, Strzelecka A, Sobala W. Mortality in the cotton industry workers: results of a cohort study. Int J Occup Med Environ Health 1999;12:143–58.
45. Schmidt C. Immune system's Toll-like receptors have good opportunity for cancer treatment. J Natl Cancer Inst 2006;98:574–5.
46. Giron-Gonzalez JA, Moral FJ, Elvira J, Garcia-Gil D, Guerrero F, Gavilan I, et al. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. Eur J Endocrinol 2000; 143:31–6.
47. Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 2000;24:627–38.
48. Pellegrini P, Contasta I, Del Beato T, Ciccone F, Berghella AM. Gender-specific cytokine pathways, targets, and biomarkers for the switch from health to adenoma and colorectal cancer. Clin Dev Immunol 2011;2011: 819724.
49. Zeeuwen PL, Boekhorst J, van den Bogaard EH, de Koning HD, van de Kerkhof PM, Saulnier DM, et al. Microbiome dynamics of human epidermis following skin barrier disruption. Genome Biol 2012;13: R101.