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Abstract

Background—Chronic inflammation and oxidative stress are thought to be involved in colorectal cancer (CRC) development. These processes may be contributed to by 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 CRC risk.

Methods—1,065 incident CRC 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 odds ratios (OR) 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 CRC risk. After testing potential interactions by various factors relevant for CRC 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 CRC 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) while 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 CRC risk among men while in women, a possible inverse association may exist.

Impact—Further studies are warranted to better clarify these preliminary observations.

Keywords

Endotoxin; Flagellin; Colorectal Cancer; European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort

Introduction

Colorectal cancer (CRC) 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 these receptors 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 CRC 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 (11). A related bacterial product is flagellin, the primary structural component of flagella and a dominant target of humoral immunity in response to infection (12). Emerging evidence suggests that an overabundance of bacterial LPS from the gut microbiota may trigger chronic inflammation and increased production of pro-inflammatory cytokines and increased reactive oxygen species (2, 13). These pro-inflammatory cytokines can activate the nuclear factor κβ (NF-κβ) pathway, which has been implicated in cell proliferation and DNA damage leading to carcinogenesis (14). Chronic inflammation has been associated with increased risk of CRC by several studies (15). Thus, hypothetically, long term exposure to the localized inflammatory responses resulting from LPS exposure may promote CRC 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

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potential associations with various disease states (16, 17). In a recent study by Ziegler *et al.* (16), 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's disease, a chronic inflammatory disease of the GI tract (18). Another line of evidence has emerged from a recent animal study which explored the intricate relationship between intestinal barrier function, microbial environment and inflammation in CRC by demonstrating that an inflammatory microenvironment promotes CRC progression in mice (19). The study highlighted that defective intestinal barrier function at tumour sites facilitates invasion of microbial products triggering inflammation and subsequent tumour growth.

While the role of microbiota in development of colorectal carcinogenesis has been explored in basic science and animal studies (19, 20), there is currently no direct epidemiologic evidence for the role of endotoxemia and gut barrier dysfunction in CRC aetiology. In the present study, we aimed to examine the association between serum LPS- and flagellin-specific immunoglobulin levels (IgA, and IgG) and risk of CRC development within a nested case-control study in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods and Materials

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 centres in 10 Western European countries (Denmark, France, Greece, Germany, Italy, 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 in detail (21). 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 sub-cohort, which is based on members of the health insurance system or state-school employees, and the Utrecht (Netherlands) sub-cohort, 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 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 (22).

In each of the study centres, fasting or non-fasting 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 (21, 22). In all countries, except Denmark and Sweden, blood was separated in the local EPIC centres and stored at the International Agency for Research on Cancer (Lyon, France;

-196°C, nitrogen vapour). In Denmark, blood samples were stored locally at -150°C under nitrogen vapour. 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 centre of Naples, where data are collected actively. Incident cancer cases were determined through record linkage with regional cancer registries (Denmark, other Italian centres, 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 enrolment and ended at the date of CRC 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 tumours 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 tumours that were overlapping or unspecified (C18.8 and C18.9). Rectal cancers were defined as tumours occurring at the recto-sigmoid junction (C19) or rectum (C20). Subjects with anal canal tumours were excluded from the study. CRC 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 CRC 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 centre (to account for centre-specific differences such as questionnaire design and blood collection procedures), 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, peri-menopausal, 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 non-melanoma skin cancer, at the time of diagnosis of the case.

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

Serum LPS- and flagellin-specific IgA and IgG levels were quantitated by ELISA at Georgia State University (Atlanta, GA, USA), as previously described (16, 17). 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 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 Ig-A-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 OD 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 intra-assay coefficient of variation (<5%) (23), our samples were analysed in singleton to minimize bio-sample volume requirement, cost and time. Inter-assay 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 (9, 24, 25). Briefly, measurements of glycated haemoglobin (HbA1c) were done on erythrocyte hemolysate using high performance liquid chromatography method (Bio-Rad Variant II instrument, Bio Lad Labor atories, Hercules, California) with intra-batch coefficient of variations of 2.5% (24). High-sensitivity C-reactive protein (hs-CRP) concentrations were measured using a high-sensitivity assay (Beckman-Coulter, Woerden, the Netherlands) on a Synchron LX-20 Pro autoanalayzer (Beckman-Coulter). The inter-assay coefficients of variation were 6.0% - 6.5% at various concentrations of hs-CRP (25).

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 antiflagellin+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 odds ratios (OR) and 95% confidence intervals (CI) of CRC, and by anatomical sub-site 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 fibre (g/day), fruits and vegetables (g/day), and red/processed

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meats (g/day) (26–31). 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 CRC risk that may be also related to LPS- and flagellin-IgA and IgG concentrations, total bacterial load and/or to colonic barrier function (32). Sex and tumour 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. alcohol intake, BMI, waist circumference, dietary fat, and hs-CRP) 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 BMI, waist circumference, dietary fat and hs-CRP by including an interaction term formed by the product of the total-antiflagellin+LPS tertile (cut-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) LPS- and flagellin exposure levels.

Conditional logistic restricted cubic spline models were used to explore possible deviation from linear relationships between each biomarker and CRC, with 4 knots specific at the median of each quartile of biomarker levels (33).

A two-tailed p-value < 0.05 was considered to be statistically significant. All statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC) 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), collectively referred to as anti-LPS+flagellin biomarkers, than their matched controls, while the concentrations of each of these serological 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 serological 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 serological 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 CRC

All models found no association between CRC and biomarkers of either anti-LPS or anti-flagellin (Supplemental Table S1). However, when analyses were stratified by sex, a significant interaction of the CRC-LPS/flagellin risk association (total-anti-flagellin+LPS, $p_{interaction} < 0.05$) was observed. Among men, there was a significant, positive association between CRC 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 vs. lowest quartiles, and a significant test for trend (p_{trend} 0.049).

In contrast, among women, there were inverse associations with CRC risk. Anti-flagellin-IgA was negatively associated with risk of CRC (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 LPS-IgA and risk of CRC with p_{trend} of 0.02. Unlike among men, the levels of total-anti-flagellin+LPS exposure were also negatively related to CRC 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 sub-sites ($p_{heterogenity} = 0.64$), 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 flagellin-IgA, 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), alcohol consumption, and dietary fat intake showed that, among men, the positive association between CRC 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, 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) (Supplemental Table S2).

Sensitivity analysis

After excluding cases that occurred during the first two 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 (Supplemental 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 LPS- and flagellin-IgA and IgG with CRC risk. No significant associations were observed with CRC risk, but sub-group analyses by sex revealed a positive association in men for LPS and flagellin markers combined, while in women the associations were inverse.

One key mechanism whereby microbiota may influence CRC development is through intestinal barrier dysfunction (34). 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 (35). A growing body of evidence supports a link between increased intestinal permeability and several GI disorders such as IBD (36), which is a known risk factor for CRC. It has been suggested that some dietary / lifestyle exposures (e.g. total fat intake, body weight) and physiological 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 (37, 38).

The impact of bacteria on the development of CRC 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 CRC (39). LPS have been suggested to be involved in CRC 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 pro-inflammatory cytokines, such as TNF-α, interleukin (IL)-1, and IL-6 (40). 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 (41, 42).

Despite a growing body of evidence from *in vitro* and *in vivo* studies on the role of the microbiome in the development of CRC, limited epidemiological studies have thus far been available to show associations between bacterial endotoxin exposure and colorectal adenomas or CRC. Two recent studies have observed a positive relationship between endotoxin and colorectal adenomas (43, 44) with the strongest associations observed for

dysplastic lesions (44). Our results showing a positive association of serum LPS and flagellin biomarkers and CRC in men are in line with the results of these studies on the role of bacteria exposure in CRC 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 LPS and flagellin levels and risk of CRC in men were stronger in higher levels of hs-CRP, waist circumference, and BMI, results which, if replicated, suggest that these factors may play a role in exacerbating the CRC promotive effects of LPS and flagellin. Also worthy of examination is the possibility that body size and inflammation may influence intestinal permeability and so lead to increased exposure to bacterial products.

Based on the observations from the above mentioned studies, an inverse association between LPS and flagellin levels and CRC 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 fibre or dust being contaminated with bacterial endotoxin (45–47) and farming industries (48), protection hypothesized to result from potential anti-carcinogenic effects of endotoxin mediated by the innate and acquired immune systems (47). 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 (49). However, these previous studies were based on occupational cohorts with high endotoxin exposures while 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 to those of previous studies looking at specific subject groups.

Two mechanisms may be involved in the differences between men and women which we observed in the associations between endotoxin and risk of CRC. First, complex interaction between the innate and adaptive immune systems are important underlying mechanisms of associations between endotoxin and carcinogenesis (50). The differences between men and women are observed 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 (51–53). 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 (54). 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 the sex-specific bacterial exposure and CRC hypothesis is required.

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

We also have several limitations in the study. First, since the gut is colonized by complex bacterial communities, elevated LPS or flagellin levels alone may not be sufficient to promote inflammation and tumour progression (43). Also, we were only able to measure IgA and IgG for either LPS or flagellin in our study, but not any other antibody isotypes. Another limitation is that we measured the LPS and 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 biological properties of these compounds is a task for future research. Moreover, bio-samples were available from 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 LPS- and flagellin-IgA and IgG, and risk of CRC. However, in sub-group analysis by sex, we found some biomarker levels to be positively associated with CRC risk among men while they were inversely associated with CRC risk among women. Further studies are warranted to elucidate the underlying mechanisms of bacterial exposure and CRC by sex as well as the sex-specific role of inflammation and immune response on CRC risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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. [PubMed: 21296855]
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. [PubMed: 18089955]
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. [PubMed: 24316595]
5. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444:1027–31. [PubMed: 17183312]
6. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World J Gastroenterol. 2014; 20:16079–94. [PubMed: 25473159]
7. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One. 2010; 5:e9085. [PubMed: 20140211]
8. Gersemann M, Wehkamp J, Stange EF. Innate immune dysfunction in inflammatory bowel disease. J Intern Med. 2012; 271:421–8. [PubMed: 22324936]
9. Aleksandrova K, Boeing H, Jenab M, Bas Bueno-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 prevention research. 2011; 4:1873–83. [PubMed: 21697276]
10. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Preliminary communication: glycated hemoglobin, diabetes, and incident colorectal cancer in men and women: a prospective analysis from the European prospective investigation into cancer-Norfolk study. Cancer Epidemiol Biomarkers Prev. 2004; 13:915–9. [PubMed: 15184246]
11. 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. [PubMed: 21270197]
12. Sanders CJ, Yu Y, Moore DA 3rd, Williams IR, Gewirtz AT. Humoral immune response to flagellin requires T cells and activation of innate immunity. Journal of immunology. 2006; 177:2810–8.
13. Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MC, et al. Energy intake is associated with endotoxemia in apparently healthy men. The American journal of clinical nutrition. 2008; 87:1219–23. [PubMed: 18469242]
14. 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. [PubMed: 18473764]
15. 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. [PubMed: 25043606]
16. 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. [PubMed: 18003793]
17. 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. [PubMed: 15388489]
18. 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. [PubMed: 15124021]

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19. 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. [PubMed: 23034650]
20. Hope ME, Hold GL, Kain R, El-Omar EM. Sporadic colorectal cancer--role of the commensal microbiota. FEMS Microbiol Lett. 2005; 244:1–7. [PubMed: 15727814]
21. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol. 1997; 26(Suppl 1):S6–14. [PubMed: 9126529]
22. 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. [PubMed: 12639222]
23. 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. [PubMed: 16439468]
24. 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. [PubMed: 18990751]
25. 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. [PubMed: 20634278]
26. 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. [PubMed: 22761771]
27. 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. The American journal of clinical nutrition. 2009; 89:1441–52. [PubMed: 19339391]
28. 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. [PubMed: 15956652]
29. 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. [PubMed: 21029790]
30. 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. The American journal of clinical nutrition. 2014; 99:139–47. [PubMed: 24225355]
31. 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. [PubMed: 17640039]
32. 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. [PubMed: 18305141]
33. Li R, Hertzmark E, Louie M, Chen L, Spiegelman D. The SAS LGTPHCURV9 Macro. 2010
34. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013; 13:800–12. [PubMed: 24132111]
35. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiological reviews. 2011; 91:151–75. [PubMed: 21248165]
36. Shen L, Su L, Turner JR. Mechanisms and functional implications of intestinal barrier defects. Dig Dis. 2009; 27:443–9. [PubMed: 19897958]

37. 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. [PubMed: 23084636]
38. 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. [PubMed: 21964884]
39. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 2014; 12:661–72. [PubMed: 25198138]
40. 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. British journal of cancer. 2009; 100:1589–602. [PubMed: 19436306]
41. 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. [PubMed: 11323673]
42. Steiner TS. How flagellin and toll-like receptor 5 contribute to enteric infection. Infect Immun. 2007; 75:545–52. [PubMed: 17118981]
43. 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. [PubMed: 23442743]
44. Lee KK, Yum KS. Association of endotoxins and colon polyp: a case-control study. J Korean Med Sci. 2012; 27:1062–5. [PubMed: 22969253]
45. 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. [PubMed: 14635236]
46. Hodgson JT, Jones RD. Mortality of workers in the British cotton industry in 1968-1984. Scand J Work Environ Health. 1990; 16:113–20. [PubMed: 2353194]
47. 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. [PubMed: 17341727]
48. 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. [PubMed: 15780775]
49. 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. [PubMed: 10465905]
50. Schmidt C. Immune system's Toll-like receptors have good opportunity for cancer treatment. J Natl Cancer Inst. 2006; 98:574–5. [PubMed: 16670380]
51. 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. [PubMed: 10870028]
52. Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev. 2000; 24:627–38. [PubMed: 10940438]
53. 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. [PubMed: 22235223]
54. 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 biology. 2012; 13:R101. [PubMed: 23153041]

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Table 1

Baseline characteristics of incident colon and rectal cancer cases and matched controls in the EPIC Cohort

Characteristics	Colon Cancer		Rectal Cancer	
	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)
Body mass index, 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)

Characteristics	Colon Cancer		Rectal Cancer	
	Cases	Controls	Cases	Controls
Dietary Intakes				
Total energy, kcal/d, median (IQR)	2066.5 (1693.2-2505.2)	2058.7 (1729.2-2453.1)	2158.6 (1726.7-2568.8)	2093.8 (1721.3-2537.8)
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)
Fibre 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)
Wome	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 haemoglobin 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)
Anti-LPS-IgA, OD, median (IQR)				

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Characteristics	Colon Cancer		Rectal Cancer	
	Cases	Controls	Cases	Controls
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 centre, hormone therapy, fasting status, and date of blood collection (within 45 days). Abbreviations: SD = standard deviation; IQR = inter-quartile range; hs-CRP = high-sensitivity C-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; LPS = lipopolysaccharide; OD = optical density; IgA = immunoglobulin A; IgG = immunoglobulin G

Table 2

ORs (95% CI) for risk of CRC 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	Quartiles*				P_{trend}
		(per 1-SD Increase) OR(95% CI)	Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
MEN							
Anti-Flic-IgA, no. Ca/Co	509/509	94/102	120/128	147/138	148/141		
SD/Cut-point	0.72	≤ 0.81	> 0.81 to ≤ 1.18	> 1.18 to ≤ 1.68	> 1.68		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	0.78	≤ 1.46	> 1.46 to ≤ 1.98	>1.98 to ≤ 2.58	> 2.58		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	0.72	≤ 1.21	> 1.21 to ≤ 1.66	> 1.66 to ≤ 2.13	> 2.13		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	0.61	≤ 1.06	> 1.06 to ≤ 1.36	> 1.36 to ≤ 1.84	> 1.84		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	1.23	≤ 2.47	> 2.47 to ≤ 3.19	>3.19 to ≤ 4.05	> 4.05		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	1.11	≤ 2.41	> 2.41 to ≤ 3.04	> 3.04 to ≤ 3.87	> 3.87		
Matching-adjusted Model [a]	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 [b]	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/Cut-point	2.00	≤ 5.13	> 5.13 to ≤ 6.35	> 6.35 to ≤ 7.73	> 7.73		
Matching-adjusted Model [a]	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 [b]	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							

Serum Immunoglobulins against LPS and Flagellin, OD		Continuous	Quartiles*				P_{trend}
		(per 1-SD Increase) OR(95% CI)	Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
Anti-Flic-IgA, no. Ca/Co	556/556	176/165	159/139	122/127	99/125		
Matching-adjusted Model [a]	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 [b]	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 [a]	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 [b]	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 [a]	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 [b]	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 [a]	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 [b]	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 [a]	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 [b]	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 [a]	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 [b]	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 [a]	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 [b]	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	

Abbreviation: OD = optical density; SD = standard deviation; OR = odds ratio; CI = confidence interval; Flic = flagellin; Ca/Co = case/control; 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

*Quartile cut-off points are same as in Table 2 and were based on the distribution of controls, expressed as optical density readings

†P_{trend} test was based on median values of each quartile

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

[b] Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, body mass index, weight circumference, physical activity, education, and total daily dietary energy consumption, fibre intake, fruits and vegetable intakes, meat and processed meat consumption)

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	Quartiles*				P_{trend}
	(per 1-SD Increase) OR(95% CI)	Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
MEN						
Anti-Flic-IgA, no. Ca/Co	298/298	55/61	79/72	79/84	85/81	
SD/Cut-point	0.74	≤ 0.85	> 0.85 to ≤ 1.23	> 1.23 to ≤ 1.72	> 1.72	
Matching-adjusted Model [a]	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[b]	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/Cut-point	0.79	≤ 1.47	> 1.47 to ≤ 2.00	> 2.00 to ≤ 2.58	> 2.58	
Matching-adjusted Model [a]	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[b]	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/Cut-point	0.72	≤ 1.23	> 1.23 to ≤ 1.70	> 1.70 to ≤ 2.20	> 2.20	
Matching-adjusted Model [a]	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[b]	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/Cut-point	0.60	≤ 1.08	> 1.08 to ≤ 1.41	> 1.41 to ≤ 1.86	< 1.86	
Matching-adjusted Model [a]	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[b]	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/Cut-point	1.26	≤ 2.50	> 2.50 to ≤ 3.26	> 3.26 to ≤ 4.11	> 4.11	
Matching-adjusted Model [a]	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[b]	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/Cut-point	1.10	≤ 2.47	> 2.47 to ≤ 3.10	> 3.10 to ≤ 3.93	> 3.93	
Matching-adjusted Model [a]	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[b]	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/Cut-point	2.00	≤ 5.28	> 5.28 to ≤ 6.46	> 6.46 to ≤ 7.91	> 7.91	
Matching-adjusted Model [a]	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[b]	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**						
Anti-Flic-IgA, no. Ca/Co	369/369	119/107	103/94	86/83	61/85	
Matching-adjusted Model [a]	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

Serum Immunoglobulins Against LPS and Flagellin, OD	Continuous	Quartiles*				P_{trend}
	(per 1-SD Increase) OR(95% CI)	Q1 OR	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
Fully-adjusted Model[b]	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[a]	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[b]	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[a]	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[b]	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[a]	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[b]	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[a]	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[b]	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[a]	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[b]	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[a]	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[b]	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

Abbreviation: OD = optical density; Flic = Flagellin; Ca/Co = Case/Control; Total Anti-Flic = anti-flagellin-IgA + anti-flagellin-IgG; Total Anti-LPS = anti-LPS-IgA + anti-LPS-IgG; Total Anti-Flic & LPS = anti-flagellin Ig-A + anti-flagellin IgG + anti-LPS IgA + anti-LPS-IgG

*Quartile cut-off points were based on the distribution of controls, expressed as optical density readings

**Quartile cut-off points are same as those in MEN

†P_{trend} test was based on median values of each quartile

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

[b]Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, body mass index, weight circumference, physical activity, education, and total daily dietary energy consumption, fibre intake, fruits and vegetable intakes, meat and processed meat consumption)

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	Quartiles*				P_{trend}
	(per 1-SD Increase) OR(95% CI)	Q1	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
MEN						
Anti-Flic-IgA, no. Ca/Co	211/211	38/42	39/49	72/60	62/60	
SD/Cut-point	0.69	≤ 0.75	> 0.75 to ≤ 1.06	> 1.06 to ≤ 1.59	> 1.59	
Matching-adjusted Model [a]	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 [b]	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/Cut-point	0.76	≤ 1.45	> 1.45 to ≤ 1.95	> 1.95 to ≤ 2.60	> 2.60	
Matching-adjusted Model [a]	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 [b]	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-point	0.71	≤ 1.14	> 1.14 to ≤ 1.57	> 1.57 to ≤ 2.02	> 2.02	
Matching-adjusted Model [a]	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 [b]	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/Cut-point	0.61	≤ 1.01	> 1.01 to ≤ 1.33	> 1.33 to ≤ 1.78	> 1.78	
Matching-adjusted Model [a]	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 [b]	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/Cut-point	1.17	≤ 2.40	> 2.40 to ≤ 3.10	> 3.10 to ≤ 3.99	> 3.99	
Matching-adjusted Model [a]	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 [b] Total	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/Cut-point	1.12	≤ 2.28	> 2.28 to ≤ 2.91	> 2.91 to ≤ 3.75	> 3.75	
Matching-adjusted Model [a]	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 [b] Total	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/Cut-point	1.98	≤ 4.95	> 4.95 to ≤ 6.11	> 6.11 to ≤ 7.55	>7.55	
Matching-adjusted Model [a]	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 [b]	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**						
Anti-Flic-IgA, no. Ca/Co	187/187	61/58	43/50	41/40	42/39	
Matching-adjusted Model [a]	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

Serum Immunoglobulins Against LPS and Flagellin, OD	Continuous	Quartiles*				P_{trend}
	(per 1-SD Increase) OR(95% CI)	Q1	Q2 OR (95% CI)	Q3 OR (95% CI)	Q4 OR (95% CI)	
Fully-adjusted Model[b]	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[a]	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[b]	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[a]	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[b]	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[a]	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[b] Total	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[a]	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[b] Total	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[a]	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[b] Total	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[a]	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[b]	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

Abbreviation: OD = optical density; Flic = Flagellin; Ca/Co = Case/Control; Total Anti-Flic = anti-flagellin-IgA + anti-flagellin-IgG; Total Anti-LPS = anti-LPS-IgA + anti-LPS-IgG; Total Anti-Flic & LPS = anti-flagellin Ig-A + anti-flagellin IgG + anti-LPS IgA + anti-LPS-IgG

*Quartile cut-off points were based on the distribution of controls, expressed as optical density readings

**Quartile cut-off points are same as those in MEN

†P_{trend} test was based on median values of each quartile

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

[b]Based on matching factors plus adjustments for established confounding factors (smoking, alcohol consumption, body mass index, weight circumference, physical activity, education, and total daily dietary energy consumption, fibre intake, fruits and vegetable intakes, meat and processed meat consumption)