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Body mass index and risk of colorectal cancer according to tumor lymphocytic infiltrate

Akiko Hanyuda[1†], Shuji Ogino[2,3,4,5†], Zhi Rong Qian[4†], Reiko Nishihara[1,4,5,6†], Mingyang Song[1,4,7,8], Kosuke Mima[4], Kentaro Inamura[4,9], Yohei Masugi[4], Kana Wu[1], Jeffrey A. Meyerhardt[4], Andrew T. Chan[4,7,8,10†], Charles S. Fuchs[3,10†], Edward L. Giovannucci[1,5,10†] and Yin Cao[1,7,8†]

[1] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA
[2] Division of MPE Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[3] Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[4] Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA
[5] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA
[6] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA
[7] Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
[8] Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA
[9] Division of Pathology, Cancer Institute, JFCR, Tokyo, Japan
[10] Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Higher body mass index (BMI), higher body adiposity and obesity have been associated with increased risk of colorectal cancer. Evidence suggests that excess energy balance may influence systemic immune and inflammatory status. Thus, we hypothesized that the positive association between BMI and colorectal cancer risk might differ according to colorectal carcinoma subtypes according to levels of histopathological lymphocytic reaction to tumor. We collected biennial questionnaire data on weight and baseline height information in two prospective cohort studies, the Nurses' Health Study (1980-2010) and the Health Professionals Follow-up Study (1986-2010). Utilizing duplication-method Cox proportional hazards regression models, we prospectively assessed the association between BMI and risk of colorectal cancer subtypes according to the degree of Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction, tumor-infiltrating lymphocytes, the overall lymphocytic reaction score, or T-cell [$CD3^+$, $CD8^+$, CD45RO (PTPRC)$^+$ or $FOXP3^+$] density in tumor tissue. Statistical significance level was adjusted for multiple hypotheses testing by Bonferroni correction. During follow up of 1,708,029 men and women (over 3,346,752 person-years), we documented 1,436 incident rectal and colon cancer cases with

Key words: body mass index, colorectal carcinoma, lymphocytic reaction, molecular pathological epidemiology

Abbreviations: BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; H&E: hematoxylin and eosin; HPFS: Health Professionals Follow-up Study; HR: hazard ratio; METS: metabolic equivalent task score; MPE: molecular pathological epidemiology; MSI: microsatellite instability; MSS: microsatellite stable; NHS: Nurses' Health Study; NSAIDs: nonsteroidal anti-inflammatory drugs; PCR: polymerase chain reaction; SD: standard deviation; TILs: tumor-infiltrating lymphocytes; WHO: World Health Organization

Additional Supporting Information may be found in the online version of this article.

A.H., S.O., Z.R.Q., and R.N. contributed equally. A.T.C., C.S.F., E.L.G., and Y.C. contributed equally.

Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols for genes (italics) and gene products (non-italics), including CD3, CD8, CRP (C-reactive protein), FOXP3 and PTPRC; all of which are described at www.genenames.org.

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Correspondence to: Edward L. Giovannucci, Department of Nutrition and Epidemiology, Harvard T.H. Chan School of Public Health, 655 Huntington Ave., Boston, MA 02215, USA, Tel: +1-617-432-4648, Fax: +1-617-432-2435, E-mail: egiovann@hsph.harvard.edu or Yin Cao, Clinical and Translational Epidemiology Unit, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02215, USA, Tel.: +1-617-415-3586, E-mail: yic576@mail.harvard.edu

available formalin-fixed paraffin-embedded tumor tissue materials and pathological immunity data. BMI was significantly associated with higher risk of overall colorectal cancer ($P_{trend} < 0.001$); however, the association of BMI with colorectal carcinoma risk did not significantly differ by the level of lymphocytic reaction or T-cell infiltration in tumor tissue status ($P_{heterogeneity} > 0.10$). BMI may be associated with risk of colorectal cancer regardless of levels of lymphocytic response to tumor.

What's new?

A vigorous immune response does not particularly hinder obesity-related cancer, according to new results. Being fat increases risk of colorectal cancer and a vigorous T-cell response can improve colorectal cancer prognosis. These authors suspected that immune cells around the tumor may suppress the oncogenic pathway induced by excess fat. Using data from the Nurses' Health Study and the Health Professionals Follow-up Study, they investigated whether the impact of BMI varied depending on the degree of lymphocytic infiltration. BMI association with colorectal cancer, they determined, did not vary with lymphocytic reaction or the density of T-cells infiltrating the tumor tissue.

Excess body adiposity, commonly measured by body mass index (BMI) contributes to the risk of colorectal cancer development.[1–4] Numerous studies suggest that obesity is characterized by chronic, low-grade inflammation.[5] Enlarged dysfunctional adipose tissue increases circulating levels of fatty acids, hormones, pro-inflammatory adipocytokines and insulin resistance, which may form a microenvironment favoring the tumor growth.[6] However, despite the role of obesity-related systemic inflammation and altered immune response in the colorectal carcinogenesis has been recognized,[7] the underlying mechanism remains uncertain.

The role of immunity for cancer causation and progression is becoming increasingly recognized.[8] It is important to elucidate the host immune status in the tumor microenvironment for better prevention and more effective therapeutic strategies.[8] Especially in colorectal carcinoma, adaptive immunity may have a pivotal role in regulating tumor evolution and proliferation.[9–11] Evidence indicates that the abundance of tumor-infiltrating T cells has been associated with microsatellite instability (MSI) and a favorable prognosis.[10,12] Truncated peptides produced by frameshift mutations due to MSI may be immunogenic and augment the host immune response.[10,12] Some epidemiological studies reported that increased BMI might promote microsatellite stable (MSS) tumor development, but not to MSI-high tumors.[13–15] Although little is known about its interrelationship between T-cell infiltrates, MSI status and other tumor molecular features, we have previously shown that greater lymphocytic reactions[16] as well as higher T-cell densities to tumor[10] are associated with longer survival of colorectal cancer patients, independent of other clinical, pathological and molecular characteristics including MSI status.

We speculate that immune cells in the tumor microenvironment may suppress the adiposity induced oncogenic pathway in colorecal cancer patients. We, therefore, hypothesize that individuals whose colonic microenvironment is enriched with low-level lymphocytic reaction might be more susceptible to the procarcinogenic effects of BMI, as compared to those with high-level lymphocytic infiltrates.

To test this hypothesis, we took a unique approach to integrate data on lifestyle factors, colorectal cancer incidence and tumor pathologic immunity status, utilizing two large U.S.-nationwide prospective cohort studies. We examined the influence of BMI on incidence of colorectal cancer according to the immunity status characterized by the pattern and intensity of lymphocytic reaction, as well as the density of tumor-infiltrating T cells.

Methods

Study population

The Nurses' Health Study (NHS) was established in 1976 with 121,701 U.S. registered female nurses who were aged 30–55 years at enrollment.[17,18] The Health Professionals Follow-up Study (HPFS) included 51,529 U.S. male health professionals who were aged 40–75 years at baseline in 1986.[17,18] In both cohorts, biennial questionnaires were used to update medical, lifestyle and other health-related information and identify newly-diagnosed cancers. This study was approved by the institutional review boards of the Harvard T.H. Chan School of Public Health and Partners Healthcare.

We excluded participants who had a history of cancer (except for nonmelanoma skin cancer) or inflammatory bowel disease and incomplete data on weight or height at baseline. We initiated follow-up on the month of return of the baseline questionnaire and ended on the month of colorectal cancer diagnosis, death from another cause or censoring date (June 1, 2010 for the NHS and January 31, 2010 for the HPFS), whichever came first. Colorectal carcinoma cases without tumor tissue data were censored from the analysis at the month of the diagnosis. We categorized BMI (kg/m^2) into 5 categories according to the World Health Organization (WHO) classification system: 18.5-<22.5, 22.5-<25, 25-<27.5, 27.5-<30,

$\geq$30 kg/m^2.[19] Because of the limited number of cases, we excluded whose BMI were less than 18.5 kg/m^2 in all analyses.

Assessment of weight and height information

In both cohorts, weight and height were self-reported at baseline and weight information was updated every two years. In the validation study, self-reported weight was highly accurate as compared to standardized measurements.[20] Trained technicians visited the individuals in a sample of cohort participants twice during the study, approximately 6 months apart to incorporate seasonal variability and measured current their weight. The Pearson correlation coefficients between self-reported and the mean of the technicians' two measurements was 0.97 in both cohorts.[20]

To examine the influence of BMI on subsequent cancer incidence, we used height reported in 1976 for women and in 1986 for men and the cumulative mean weight which was the mean of all available weight data up to the start of each 2-year follow-up period.

Assessment of colorectal cancer cases

In both cohorts, incident colorectal cancer cases were ascertained by biennial questionnaire and the use of the National Death Index. We estimate ascertaining >90% of incidence colorectal cancers and >98% of fatal cases. Study physicians, blinded to exposure information, reviewed all medical and pathological records to confirm the disease diagnosis and to retrieve information on tumor anatomic location, disease stage and histological type of cancer.

Histopathological, immunohistochemical and tumor molecular analyses

Hematoxylin and eosin (H&E)-stained tissue sections from all colorectal carcinoma cases were reviewed under a light microscope by the study pathologist (S.O.). Tumor differentiation was classified as well/moderate *versus* poor.[21] The extent of mucinous component was evaluated and tumors were classified as negative *versus* 1–49% mucinous component *versus* $\geq$50% mucinous component, as previously described.[22] Four components of lymphocytic reactions [Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction and tumor-infiltrating lymphocytes (TILs)] were evaluated as previously described,[16] and each component was scored as low (0), intermediate (1+), moderate (2+) or marked (3+). Because of the limited cases categorized as moderate or marked, we combined these categories as "high" in our primary hypothesis testing analyses. In addition, we used the overall lymphocytic reaction score (0–12) as the sum of scores for the above four reaction components and divided cases into 3 groups: low (scores of 0–1), intermediate (scores of 2–5) and high (scores of 6–12).

To measure the densities of CD3$^+$, CD8$^+$, CD45RO (PTPRC)$^+$ and FOXP3$^+$ T cells in tumor tissue, we constructed a tissue microarray,[17] and conducted immunohistochemistry and image analysis using automated scanning microscope and the Ariol image analysis system (Genetix, San Jose, CA).[10] We classified each of the T-cell densities (cells/mm^2) into quartiles (Q1, the lowest, to Q4, the highest) with cut-off points described in Table 3. In our primary analyses, we divided cases into two groups: low (Q1–Q2) or high (Q3–Q4). In our exploratory analyses, we used T-cell density score [4–16 (or 3–12)] as the sum of the quartile scores (assigned as follows: 1 for Q1, 2 for Q2, 3 for Q3 and 4 for Q4) of the four T-cell subsets (or three, excluding FOXP3$^+$ cell density).

DNA was extracted from tumor tissue and tumor MSI status was determined by polymerase chain reaction (PCR) of microsatellites as previously described.[23]

Statistical analysis

We calculated age-standardized mean values and proportions for potential confounders according to the categories of BMI. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) using the most updated available information for all variables prior to each two-year follow-up period. If participants missed weight or covariates information in biennial questionnaires, we used most recent available information from the past questionnaires. Trend tests across categories of BMI were performed by using the median value for each category as a continuous variable. Multivariable HRs were adjusted for age, family history of colorectal cancer in any first-degree relative (yes or no), history of diabetes (yes or no), history of colonoscopy or sigmoidoscopy (yes or no), smoking status (never, <5, 5–19, 20–39 and 40+ pack-years), physical activity [quintiles of metabolic equivalent task score (METS) per week], red and processed meat intake (quintiles of servings per day), alcohol consumption (0, <5, 5-<15, 15-<30, 30+ gm per day), current multivitamin use (yes or no), regular use of aspirin (yes or no), regular use of nonsteroidal anti-inflammatory drugs [NSAIDs (yes or no)], total energy intake (quintiles of calories per day), folate intake (quintiles of μg per day), calcium intake (quintiles of mg per day) and Alternate Healthy Eating Index (AHEI)−2010 (quintiles of the overall AHEI-2010 score without alcohol intake). For female participants, we additionally adjusted for menopause/postmenopausal hormone-replacement therapy use status (premenopausal, postmenopausal never hormone use, postmenopausal past hormone use or postmenopausal current hormone use). All variables were defined time-varying to take into account potential changes over follow-up time. We did not observe significant heterogeneity between the NHS and the HPFS for the association of BMI and overall colorectal cancer risk ($P_{\text{heterogeneity}} > 0.05$ for Cochran's Q test); thus, we primarily used a combined cohort of women and men to maximize statistical power. As secondary analyses, we examined the relation between BMI and colorectal cancer risk according to intensities of lymphocytic reaction in each cohort, and confirmed consistency in results between the two cohorts.

Our primary hypothesis testing was the heterogeneity test on the subtype-specific HR estimates according to levels of

lymphocytic reaction. To account for multiple hypothesis testing for the four lymphocytic components (Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction and TILs) and the overall lymphocytic reaction score, we used Bonferroni correction to adjust the statistical significance level to α of 0.01 (=0.05/5). We examined whether each subtype-specific association had an increasing or decreasing ordinal trend by levels of lymphocytic reaction using a likelihood ratio test with one degree of freedom.[24,25] The statistical significance of this trend was represented as $P_{\text{heterogeneity}}$. In a subset of cases with tissue microarray data, we analyzed whether the association between BMI and colorectal carcinoma risk might differ by density of each T cell ($CD3^+$, $CD8^+$, $CD45RO^+$ or $FOXP3^+$) or the sum of T-cell density scores with the four (or three) markers.

In our exploratory analyses, we evaluated the association between BMI and colorectal carcinoma risk according to MSI status, tumor differentiation or the extent of mucinous components.

All other assessments were secondary analyses. All *p*-values were two-sided. We recognized the potential or type 1 error due to multiple comparisons and interpreted our data cautiously in addition to the use of Bonferroni correction. All analyses were performed using SAS software (Version 9.3, SAS Institute, Cary, NC).

Results

Table 1 shows the age-standardized characteristics of the NHS (1980–2010) and the HPFS (1986–2010) participants according to BMI categories. In both cohorts, BMI was positively associated with history of diabetes, regular use of NSAIDs or aspirin and red and processed meat intake; whereas inversely related to current use of multivitamin, alcohol, calcium and folate intakes and physical activity. Postmenopausal women with high BMI were less likely to use menopausal hormone therapy. There were no substantial differences in demographic or clinical features between cases with and without tumor immunity data (Table S1, Supporting Information).

We documented 1,436 colorectal cancer cases over 3,346,752 person-years follow-up with available tissue suitable for immunity analyses. Consistent with our prior analyses over earlier follow-up,[26,27] BMI was significantly associated with higher risk of overall colorectal cancer ($P_{\text{trend}} < 0.001$) (Table 2).

Our primary aim was to assess the heterogeneity of the association between BMI and incidence of colorectal carcinoma according to the degree of lymphocytic reactions to tumor. In the pooled cohort, the association of BMI with risk of colorectal cancer did not statistically significantly differ by the level of lymphocytic reaction pattern (Crohn's-like reaction, peritumoral reaction, intratumor periglandular reaction or TILs) or the overall lymphocytic score ($P_{\text{heterogeneity}} > 0.10$, with the adjusted α level of 0.01) (Table 2). Although statistical power was limited in certain subtypes, in general there appeared to be positive associations of BMI with risks of most colorectal cancer subtypes according to levels of lymphocytic reaction patterns.

We additionally subclassified colorectal cancer according to the density of each of the four T-cell ($CD3^+$, $CD8^+$, $CD45RO^+$ or $FOXP3^+$) subsets within tumor tissues, or combined T-cell density score. Similar to the results on lymphocytic reaction pattern, there was no significant heterogeneous association between BMI and colorectal carcinoma risk according to the density of T cells, or combined T-cell score ($P_{\text{heterogeneity}} > 0.60$) (Table 3).

As exploratory analyses, we investigated the association between BMI and colorectal cancer risk according to MSI status, tumor differentiation or the extent of mucinous components and found no significant heterogeneity according to these subtypes in men, women or the combined cohort ($P_{\text{heterogeneity}} > 0.03$) (Tables 4 and S6, Supporting Information).

Discussion

This study represents a unique analysis to integrate data on BMI, cancer incidence and pathologic examination of lymphocytic reaction to tumor, leveraging the two large prospective cohort studies. We observed that the positive association between BMI and colorectal cancer incidence did not significantly differ according to tumor lymphocytic reaction pattern. Additionally, we did not find substantial differences in the association by T-cell density or tumor MSI status. Our results suggest that adiposity may be associated with a higher risk of colorectal carcinoma regardless of tumor lymphocytic infiltrate patterns analyzed in this study.

Although a growing body of evidence suggests that adipokine pathophysiology and systemic inflammation may be a putative mediator of the causal link between excess body fat and colorectal cancer development,[6] none of the previous studies has yet examined the association of obesity with risk of colorectal cancer according to levels of immune response to tumor. Leptin and adiponectin are the types of adipokines relevant for tumorigenesis in obese state.[28] Potentially, leptin has an oncogenic role whereas adiponectin inhibits tumor growth[28]; however, recent meta-analyses examining associations between serum leptin/adiponectin concentration and colorectal cancer risk remain inconclusive.[29,30] Our previous study has shown that plasma adiponectin was significantly associated with reduced risk of colorectal cancer in men, but not among women[31] whereas plasma soluble leptin receptor, a bioactive modulator of free leptin, did not reach significant levels for both women and men.[31] In addition, despite an early epidemiological study showed a significant association of C-reactive protein (CRP) levels with colorectal cancer,[32] subsequent studies reported modest correlations[33,34] or null findings.[35] Of note, these inconsistent results may arise because biological effects of inflammation biomarkers or adipokines may depend not only circulating concentrations but also its form and the tissue-specific expression in tumor

Table 1. Age-standardized characteristics of the NHS (1980–2010) and the HPFS (1986–2010) participants according to body mass index (person-years).

	Body mass index (kg/m^2)									
	NHS					HPFS				
Characteristics	**18.5–22.5**	**22.5–24.9**	**25–27.5**	**27.5–29.9**	**≥30**	**18.5–22.5**	**22.5–24.9**	**25–27.5**	**27.5–29.9**	**≥30**
Participants, person-years	352,576	323,576	228,396	135,304	173,152	59,433	161,813	154,675	73,419	45,684
Age (year)[1]	57.6 (11.3)	60.0 (10.8)	61.3 (10.6)	62.1 (10.4)	61.2 (10.2)	63.7 (12.1)	63.4 (11.4)	63.6 (10.9)	63.4 (10.3)	63.0 (9.9)
Family history of cancer (%)	12.5	12.9	13.1	13.2	13.4	12.1	12.0	12.3	12.2	12.5
History of diabetes (%)	2.2	3.4	6.5	10.6	20.4	3.9	4.8	6.6	9.9	18.0
Body mass index (kg/m^2)	21.0 (1.0)	23.7 (0.7)	26.2 (0.7)	28.7 (0.7)	34.0 (3.8)	21.5 (0.9)	23.8 (0.7)	26.1 (0.7)	28.5 (0.7)	32.9 (4.0)
Postmenopause (%)	73.7	77.1	78.6	79.5	78.6	-	-	-	-	-
Current hormone therapy use (%)	32.1	30.2	27.1	24.9	20.3	-	-	-	-	-
History of colonoscopy/ sigmoidoscopy (%)	36.7	38.0	37.6	38.0	36.9	51.7	52.5	51.5	51.2	51.2
Current use of multivitamin (%)	52.7	51.2	50.4	49.4	47.6	50.8	48.3	45.4	43.2	42.0
Regular use of NSAIDs (%)	14.3	17.0	18.4	19.9	21.5	11.3	13.7	15.3	17.5	19.3
Regular use of aspirins (%)	32.2	33.5	35.3	36.2	37.8	41.2	45.6	47.6	49.1	49.7
Pack-year among ever smokers	23.8 (20.5)	23.7 (20.1)	24.2 (20.2)	24.0 (20.1)	23.4 (20.5)	23.6 (19.9)	23.1 (18.7)	24.9 (18.8)	26.7 (19.6)	28.1 (20.2)
Total calorie (kcal/d)	1665 (442)	1662 (435)	1667 (441)	1682 (447)	1716 (460)	2002 (544)	1967 (544)	1964 (561)	1977 (571)	2001 (586)
Alcohol intake (g/d)	7.5 (10.2)	6.7 (9.6)	5.6 (9.0)	4.7 (8.3)	3.4 (7.2)	10.3 (13.4)	11.2 (13.5)	11.3 (14.1)	11.2 (14.5)	9.9 (14.3)
Physical activity, METS (week)	19.9 (20.7)	17.6 (17.8)	15.5 (15.6)	13.8 (14.7)	11.5 (12.7)	34.0 (33.3)	32.8 (29.3)	29.6 (28.4)	26.7 (25.9)	22.0 (23.5)
Red and processed meat servings (week)	6.3 (3.6)	6.5 (3.6)	6.7 (3.6)	6.9 (3.6)	7.4(3.9)	5.5 (4.5)	5.8 (4.2)	6.6 (4.4)	7.1 (4.5)	7.8 (4.8)
Calcium (mg/d)	927 (367)	926 (353)	919 (347)	914 (344)	896 (340)	948 (389)	935 (378)	918 (368)	920 (366)	927 (374)
Folate (μg/d)	427 (217)	421 (212)	416 (206)	411 (217)	405 (205)	565 (265)	551 (257)	528 (250)	512 (237)	507 (243)
Alternate Healthy Eating Index (AHEI)−2010[2]	46.1 (9.9)	46.0 (9.5)	45.9 (9.3)	45.7 (9.1)	44.8 (9.1)	49.7 (11.0)	48.9 (10.2)	47.6 (9.8)	46.9 (9.5)	46.2 (9.6)

[1]All values other than age have been directly standardized to age distribution (in 5-year age group) of all the participants. Mean [standard deviation (SD)] was presented for continuous variables.
[2]Without alcohol intake.

Table 2. Body mass index and risk of colorectal cancer overall and by components of lymphocytic reaction[1] (Pooled)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$[2]
Total colorectal cancer							
Person-years	812,890	951,721	747,733	407,240	427,169		
Cases no. ($n = 1436$)	226	403	379	207	221		
Age-adjusted HR (95% CI)	1 [Ref]	1.22 (1.03, 1.44)	1.36 (1.15, 1.61)	1.39 (1.15, 1.69)	1.61 (1.33, 1.94)	<0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.22 (1.03, 1.44)	1.34 (1.13, 1.59)	1.36 (1.12, 1.65)	1.57 (1.29, 1.91)	<0.001	
Crohn's-like lymphoid reaction							
Low (0)							
Cases, no. ($n = 887$)	138	261	230	120	138		
Age-adjusted HR (95% CI)	1 [Ref]	1.32 (1.07, 1.63)	1.38 (1.11, 1.71)	1.34 (1.04, 1.72)	1.65 (1.30, 2.10)	<0.001	0.14
Multivariable HR (95% CI)[3]	1 [Ref]	1.32 (1.07, 1.62)	1.37 (1.10, 1.70)	1.32 (1.02, 1.70)	1.62 (1.27, 2.08)	<0.001	0.15
Intermediate (1+)							
Cases, no. ($n = 202$)	34	52	55	35	26		
Age-adjusted HR (95% CI)	1 [Ref]	1.00 (0.64, 1.54)	1.24 (0.80, 1.92)	1.49 (0.92, 2.41)	1.15 (0.69, 1.94)	0.23	
Multivariable HR (95% CI)[3]	1 [Ref]	0.99 (0.64, 1.54)	1.23 (0.79, 1.91)	1.44 (0.89, 2.34)	1.13 (0.67, 1.92)	0.28	
High (2+ - 3+)							
Cases, no. ($n = 84$)	9	18	25	13	19		
Age-adjusted HR (95% CI)	1 [Ref]	1.40 (0.63, 3.14)	2.23 (1.03, 4.83)	2.13 (0.91, 5.02)	3.44 (1.55, 7.64)	<0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.43 (0.64, 3.21)	2.25 (1.04, 4.87)	2.10 (0.89, 4.96)	3.42 (1.53, 7.62)	<0.001	
Peritumoral lymphocytic reaction							
Low (0)							
Cases, no. ($n = 204$)	23	58	62	25	36		
Age-adjusted HR (95% CI)	1 [Ref]	1.77 (1.08, 2.88)	2.17 (1.34, 3.54)	1.69 (0.95, 2.99)	2.60 (1.54, 4.41)	0.001	0.94
Multivariable HR (95% CI)[3]	1 [Ref]	1.77 (1.09, 2.89)	2.16 (1.33, 3.52)	1.65 (0.93, 2.93)	2.52 (1.48, 4.29)	0.002	0.96
Intermediate (1+)							
Cases, no. ($n = 998$)	169	284	261	140	144		
Age-adjusted HR (95% CI)	1 [Ref]	1.15 (0.95, 1.40)	1.27 (1.04, 1.55)	1.28 (1.02, 1.61)	1.42 (1.14, 1.78)	0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.15 (0.94, 1.39)	1.25 (1.03, 1.53)	1.25 (0.99, 1.58)	1.39 (1.10, 1.75)	0.005	
High (2+ - 3+)							
Cases, no. ($n = 224$)	34	56	52	41	41		
Age-adjusted HR (95% CI)	1 [Ref]	1.09 (0.71, 1.68)	1.15 (0.74, 1.79)	1.65 (1.04, 2.62)	1.82 (1.15, 2.88)	0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.10 (0.71, 1.69)	1.15 (0.74, 1.79)	1.62 (1.02, 2.57)	1.79 (1.12, 2.84)	0.002	

Table 2. Body mass index and risk of colorectal cancer overall and by components of lymphocytic reaction (Pooled) (Continued)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$ [2]
Intratumoral periglandular reaction							
Low (0)							
Cases, no. ($n = 190$)	25	56	53	18	38		
Age-adjusted HR (95% CI)	1 [Ref]	1.54 (0.96, 2.48)	1.73 (1.06, 2.81)	1.08 (0.59, 2.00)	2.45 (1.47, 4.08)	0.003	0.78
Multivariable HR (95% CI)[3]	1 [Ref]	1.55 (0.96, 2.50)	1.71 (1.05, 2.78)	1.06 (0.58, 1.97)	2.38 (1.42, 3.97)	0.006	0.77
Intermediate (1+)							
Cases, no. ($n = 1060$)	177	297	279	157	150		
Age-adjusted HR (95% CI)	1 [Ref]	1.15 (0.95, 1.38)	1.28 (1.05, 1.55)	1.35 (1.09, 1.69)	1.40 (1.13, 1.75)	<0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.14 (0.94, 1.38)	1.26 (1.04, 1.53)	1.32 (1.06, 1.65)	1.37 (1.09, 1.72)	0.003	
High (2+ - 3+)							
Cases, no. ($n = 182$)	24	46	47	32	33		
Age-adjusted HR (95% CI)	1 [Ref]	1.30 (0.79, 2.15)	1.57 (0.95, 2.59)	1.98 (1.16, 3.39)	2.19 (1.29, 3.71)	<0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.31 (0.79, 2.16)	1.56 (0.95, 2.57)	1.92 (1.12, 3.29)	2.13 (1.25, 3.64)	0.001	
Tumor-infiltrating lymphocytes							
Low (0)							
Cases, no. ($n = 1074$)	170	306	280	155	163		
Age-adjusted HR (95% CI)	1 [Ref]	1.22 (1.01, 1.47)	1.31 (1.08, 1.59)	1.38 (1.10, 1.72)	1.60 (1.29, 1.98)	<0.001	0.86
Multivariable HR (95% CI)[3]	1 [Ref]	1.21 (1.00, 1.47)	1.29 (1.06, 1.57)	1.34 (1.07, 1.67)	1.55 (1.24, 1.95)	<0.001	0.87
Intermediate (1+)							
Cases, no. ($n = 213$)	31	60	52	29	41		
Age-adjusted HR (95% CI)	1 [Ref]	1.35 (0.87, 2.09)	1.44 (0.92, 2.26)	1.42 (0.85, 2.37)	2.06 (1.29, 3.31)	0.004	
Multivariable HR (95% CI)[3]	1 [Ref]	1.34 (0.86, 2.08)	1.42 (0.90, 2.24)	1.39 (0.83, 2.33)	2.02 (1.25, 3.25)	0.007	
High (2+ - 3+)							
Cases, no. ($n = 145$)	24	36	45	23	17		
Age-adjusted HR (95% CI)	1 [Ref]	1.09 (0.64, 1.83)	1.63 (0.98, 2.69)	1.54 (0.86, 2.74)	1.16 (0.62, 2.17)	0.31	
Multivariable HR (95% CI)[3]	1 [Ref]	1.09 (0.64, 1.83)	1.61 (0.97, 2.66)	1.50 (0.84, 2.68)	1.13 (0.60, 2.13)	0.37	
Overall lymphocytic reaction score							
Low (scores of 0-1)							
Cases, no. ($n = 218$)	28	62	61	26	41		
Age-adjusted HR (95% CI)	1 [Ref]	1.50 (0.96, 2.36)	1.71 (1.09, 2.71)	1.39 (0.81, 2.39)	2.40 (1.48, 3.90)	0.001	0.70
Multivariable HR (95% CI)[3]	1 [Ref]	1.51 (0.96, 2.37)	1.72 (1.09, 2.71)	1.37 (0.80, 2.35)	2.35 (1.44, 3.83)	0.002	0.72

Table 2. Body mass index and risk of colorectal cancer overall and by components of lymphocytic reaction (Pooled) (Continued)

	Body mass index (kg/m^2)						
	18.5–22.5	**22.5–24.9**	**25–27.5**	**27.5–29.9**	**≥30**	P_{trend}	$P_{heterogeneity}$[2]
Intermediate (scores of 2-5)							
Cases, no. ($n = 847$)	140	245	219	121	122		
Age-adjusted HR (95% CI)	1 [Ref]	1.21 (0.98, 1.50)	1.30 (1.04, 1.61)	1.32 (1.03, 1.69)	1.42 (1.11, 1.81)	0.005	
Multivariable HR (95% CI)[3]	1 [Ref]	1.21 (0.98, 1.49)	1.28 (1.03, 1.60)	1.30 (1.01, 1.67)	1.40 (1.08, 1.80)	0.009	
High (scores of 6-12)							
Cases, no. ($n = 106$)	13	22	30	21	20		
Age-adjusted HR (95% CI)	1 [Ref]	1.14 (0.57, 2.28)	1.79 (0.92, 3.45)	2.35 (1.17, 4.72)	2.47 (1.23, 5.00)	0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.18 (0.59, 2.36)	1.79 (0.93, 3.47)	2.30 (1.14, 4.63)	2.44 (1.20, 4.94)	0.002	

[1]Each component of lymphocytic reactions [Crohn's-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction and tumor-infiltrating lymphocytes (TILs)] was evaluated as low (0), intermediate (1+) and high (2+ - 3+). The overall lymphocytic reaction score (0–12) was the sum of scores for the above four reaction components. In this analysis, overall lymphocytic reaction score was divided into 3 groups: low (scores of 0–1), intermediate (scores of 2–5) and high (scores of 6–12).

[2]Adjusted for family history of colorectal cancer (yes/no), history of diabetes (yes/no), history of colonoscopy/sigmoidoscopy (yes/no), smoking in pack-years (never, <5,5–19, 20–39, 40+ pack-years), physical activity [quintiles of metabolic equivalent task score (METS) per week], red and processed meat intake (quintiles of servings per day), alcohol consumption (0, <5, 5-<15, 15-<30, 30+ gm per day), current multivitamin use (yes/no), regular use of aspirin (yes/no), regular use of nonsteroidal anti-inflammatory drugs [NSAIDs (yes/no)], total energy intake (quintiles of calories per day), folate intake (quintiles of μg per day), calcium intake (quintiles of mg per day) and Alternate Healthy Eating Index (AHEI)−2010 (quintiles of the overall AHEI-2010 score without alcohol intake). For women, we additionally adjusted for menopause/postmenopausal hormone use status (premenopausal, postmenopausal never use, postmenopausal past use or postmenopausal current hormone use).

[3]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to the subtyping marker, using a likelihood ratio test with one degree of freedom and the statistical significance of this trend was presented as $P_{heterogeneity}$.

Table 3. Body mass index and risk of colorectal cancer by tumor-infiltrating T-cell subset density[1] (Pooled)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$[2]
CD3$^+$ cells							
Low (Q1 to Q2)							
Cases no. ($n = 345$)	50	104	96	42	53		
Age-adjusted HR (95% CI)	1 [Ref]	1.52 (1.08, 2.15)	1.71 (1.21, 2.43)	1.40 (0.92, 2.12)	1.79 (1.21, 2.65)	0.01	0.74
Multivariable HR (95% CI)[3]	1 [Ref]	1.54 (1.09, 2.16)	1.69 (1.19, 2.40)	1.35 (0.89, 2.05)	1.71 (1.15, 2.55)	0.03	0.79
High (Q3 to Q4)							
Cases, no. ($n = 345$)	55	96	86	53	55		
Age-adjusted HR (95% CI)	1 [Ref]	1.26 (0.90, 1.77)	1.37 (0.97, 1.93)	1.56 (1.07, 2.29)	1.70 (1.16, 2.47)	0.003	
Multivariable HR (95% CI)[3]	1 [Ref]	1.26 (0.90, 1.77)	1.34 (0.95, 1.90)	1.49 (1.01, 2.19)	1.59 (1.08, 2.34)	0.01	
CD8$^+$ cells							
Low (Q1 to Q2)							
Cases, no. ($n = 340$)	53	99	85	45	58		
Age-adjusted HR (95% CI)	1 [Ref]	1.40 (1.00, 1.96)	1.46 (1.03, 2.08)	1.40 (0.94, 2.09)	1.81 (1.24, 2.63)	0.005	0.86
Multivariable HR (95% CI)[3]	1 [Ref]	1.40 (0.99, 1.96)	1.44 (1.01, 2.04)	1.35 (0.90, 2.03)	1.70 (1.16, 2.50)	0.02	0.85
High (Q3 to Q4)							
Cases, no. ($n = 337$)	53	95	94	47	48		
Age-adjusted HR (95% CI)	1 [Ref]	1.30 (0.92, 1.82)	1.55 (1.10, 2.19)	1.46 (0.98, 2.18)	1.58 (1.07, 2.34)	0.01	
Multivariable HR (95% CI)[3]	1 [Ref]	1.30 (0.93, 1.83)	1.53 (1.08, 2.16)	1.39 (0.93, 2.08)	1.50 (1.00, 2.24)	0.04	
CD45RO$^+$ cells							
Low (Q1 to Q2)							
Cases, no. ($n = 349$)	47	103	98	47	54		
Age-adjusted HR (95% CI)	1 [Ref]	1.51 (1.07, 2.15)	1.69 (1.19, 2.41)	1.54 (1.02, 2.32)	1.89 (1.28, 2.81)	0.004	0.69
Multivariable HR (95% CI)[3]	1 [Ref]	1.52 (1.07, 2.15)	1.67 (1.17, 2.38)	1.48 (0.98, 2.24)	1.81 (1.21, 2.70)	0.01	0.70
High (Q3 to Q4)							
Cases, no. ($n = 354$)	62	102	86	47	57		
Age-adjusted HR (95% CI)	1 [Ref]	1.27 (0.92, 1.75)	1.35 (0.97, 1.88)	1.32 (0.90, 1.94)	1.58 (1.10, 2.28)	0.02	
Multivariable HR (95% CI)[3]	1 [Ref]	1.29 (0.93, 1.77)	1.34 (0.96, 1.87)	1.27 (0.87, 1.88)	1.52 (1.04, 2.20)	0.04	

Table 3. Body mass index and risk of colorectal cancer by tumor-infiltrating T-cell subset density (Pooled) (Continued)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$[2]
FOXP3$^+$ cells							
Low (Q1 to Q2)							
Cases, no. ($n = 339$)	59	95	83	41	61		
Age-adjusted HR (95% CI)	1 [Ref]	1.17 (0.84, 1.62)	1.23 (0.87, 1.73)	1.15 (0.77, 1.72)	1.74 (1.21, 2.49)	0.005	0.82
Multivariable HR (95% CI)[3]	1 [Ref]	1.17 (0.84, 1.63)	1.22 (0.86, 1.72)	1.11 (0.74, 1.66)	1.68 (1.16, 2.44)	0.01	0.84
High (Q3 to Q4)							
Cases, no. ($n = 328$)	48	94	90	49	47		
Age-adjusted HR (95% CI)	1 [Ref]	1.47 (1.04, 2.09)	1.71 (1.20, 2.44)	1.69 (1.13, 2.53)	1.64 (1.09, 2.47)	0.01	
Multivariable HR (95% CI)[3]	1 [Ref]	1.49 (1.05, 2.12)	1.71 (1.19, 2.45)	1.67 (1.11, 2.51)	1.60 (1.06, 2.43)	0.03	
T-cell density score (3 to 12)							
Low (scores of 3-5)							
Cases, no. ($n = 185$)	31	53	42	27	32		
Age-adjusted HR (95% CI)	1 [Ref]	1.22 (0.78, 1.91)	1.18 (0.73, 1.90)	1.37 (0.81, 2.31)	1.62 (0.98, 2.68)	0.06	0.72
Multivariable HR (95% CI)[3]	1 [Ref]	1.23 (0.78, 1.92)	1.18 (0.73, 1.89)	1.32 (0.78, 2.25)	1.56 (0.93, 2.59)	0.10	0.73
Intermediate (scores of 6-9)							
Cases, no. ($n = 368$)	57	107	103	48	53		
Age-adjusted HR (95% CI)	1 [Ref]	1.38 (1.00, 1.91)	1.62 (1.16, 2.25)	1.43 (0.97, 2.11)	1.63 (1.12, 2.38)	0.01	
Multivariable HR (95% CI)[3]	1 [Ref]	1.39 (1.00, 1.93)	1.58 (1.13, 2.20)	1.36 (0.92, 2.02)	1.54 (1.04, 2.26)	0.05	
High (scores of 10-12)							
Cases, no. ($n = 152$)	22	44	38	22	26		
Age-adjusted HR (95% CI)	1 [Ref]	1.49 (0.89, 2.50)	1.58 (0.92, 2.69)	1.63 (0.90, 2.97)	1.97 (1.11, 3.49)	0.02	
Multivariable HR (95% CI)[3]	1 [Ref]	1.50 (0.90, 2.53)	1.58 (0.92, 2.69)	1.58 (0.87, 2.89)	1.89 (1.06, 3.37)	0.04	
T-cell density score (4 to 16)							
Low (scores of 4-7)							
Cases, no. ($n = 178$)	32	48	45	22	31		
Age-adjusted HR (95% CI)	1 [Ref]	1.10 (0.70, 1.73)	1.25 (0.78, 1.98)	1.15 (0.66, 1.99)	1.59 (0.96, 2.63)	0.07	0.83
Multivariable HR (95% CI)[3]	1 [Ref]	1.10 (0.70, 1.73)	1.23 (0.77, 1.97)	1.11 (0.64, 1.94)	1.53 (0.92, 2.55)	0.11	0.85

Table 3. Body mass index and risk of colorectal cancer by tumor-infiltrating T-cell subset density (Pooled) (Continued)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$[2]
Intermediate (scores of 8-12)							
Cases, no. ($n = 401$)	59	116	113	54	59		
Age-adjusted HR (95% CI)	1 [Ref]	1.42 (1.03, 1.95)	1.68 (1.22, 2.31)	1.51 (1.04, 2.20)	1.72 (1.19, 2.47)	0.004	
Multivariable HR (95% CI)[3]	1 [Ref]	1.43 (1.04, 1.96)	1.66 (1.20, 2.29)	1.46 (1.00, 2.13)	1.64 (1.13, 2.38)	0.02	
High (scores of 13-16)							
Cases, no. ($n = 127$)	20	38	28	19	22		
Age-adjusted HR (95% CI)	1 [Ref]	1.47 (0.85, 2.54)	1.31 (0.73, 2.35)	1.58 (0.84, 2.98)	1.86 (1.01, 3.42)	0.06	
Multivariable HR (95% CI)[3]	1 [Ref]	1.48 (0.86, 2.57)	1.30 (0.73, 2.34)	1.50 (0.79, 2.85)	1.79 (0.97, 3.32)	0.09	

[1]We classified each T-cell subset density (cells/mm^2) into quartiles: Q1, Q2, Q3 or Q4 (assigned as follows: 1 for Q1, 2 for Q2, 3 for Q3 and 4 for Q4). Cut-off density (cells/mm^2) of each Q1, Q2, Q3 and Q4 was follows: $0 \leq 86.27$, $86.27 \leq 244.97$, $244.97 \leq 580.72$, 580.72- for $CD3^+$; $0 \leq 76.68$, $76.68 \leq 236.65$, $236.65 \leq 646.4$, 646.4- for $CD8^+$ cells; $0 \leq 158.66$, $158.66 \leq 376.97$, $376.97 \leq 726.55$, 726.55- for $CD45RO^+$ cells; and $0 \leq 13.56$, $13.56 \leq 26.36$, $26.36 \leq 48.07$, 48.07- for $FOXP3^+$ cells.
T-cell density score (3–12) was the sum of scores for the above three T-cell ($CD3^+$, $CD8^+$ and $CD45RO^+$) density subsets. In this analysis, T-cell density score was divided into three groups: low (scores of 3–5), intermediate (scores of 6–9) and high (scores of 10–12).
T-cell density score (4–16) was the sum of scores for the above four T-cell ($CD3^+$, $CD8^+$, $CD45RO^+$ and $FOXP3^+$) density subsets. In this analysis, T-cell density score was divided into three groups: low (scores of 4–7), intermediate (scores of 8–12) and high (scores of 13–16).
[2]Adjusted for the same set of covariates as in Table 2.
[3]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to the density of T cells, using a likelihood ratio test with one degree of freedom and the statistical significance of this trend was presented as $P_{heterogeneity}$.

Table 4. Body mass index and risk of colorectal cancer by microsatellite instability, tumor differentiation or mucinous component[1] (Pooled)

	Body mass index (kg/m^2)						
	18.5–22.5	22.5–24.9	25–27.5	27.5–29.9	≥30	P_{trend}	$P_{heterogeneity}$[2]
Microsatellite instability (MSI)							
Microsatellite stable (MSS)							
Cases, no. ($n = 1093$)	161	314	298	165	155		
Age-adjusted HR (95% CI)	1 [Ref]	1.30 (1.07, 1.58)	1.47 (1.21, 1.79)	1.55 (1.24, 1.93)	1.58 (1.27, 1.98)	<0.001	0.70
Multivariable HR (95% CI)[3]	1 [Ref]	1.29 (1.07, 1.57)	1.45 (1.19, 1.76)	1.49 (1.19, 1.87)	1.52 (1.21, 1.91)	<0.001	0.71
MSI-high							
Cases, no. ($n = 210$)	39	55	52	28	36		
Age-adjusted HR (95% CI)	1 [Ref]	1.02 (0.67, 1.55)	1.12 (0.74, 1.71)	1.08 (0.66, 1.76)	1.43 (0.91, 2.26)	0.12	
Multivariable HR (95% CI)[3]	1 [Ref]	1.02 (0.67, 1.54)	1.11 (0.73, 1.70)	1.04 (0.64, 1.71)	1.37 (0.86, 2.18)	0.18	
Tumor differentiation							
Well/moderate							
Cases, no. ($n = 1366$)	205	368	373	207	213		
Age-adjusted HR (95% CI)	1 [Ref]	1.21 (1.01, 1.44)	1.45 (1.21, 1.72)	1.51 (1.24, 1.84)	1.70 (1.40, 2.07)	<0.001	0.04
Multivariable HR (95% CI)[3]	1 [Ref]	1.20 (1.01, 1.43)	1.42 (1.19, 1.69)	1.47 (1.20, 1.79)	1.65 (1.35, 2.02)	<0.001	0.04
Poor							
Cases, no. ($n = 149$)	29	51	30	20	19		
Age-adjusted HR (95% CI)	1 [Ref]	1.28 (0.81, 2.03)	0.91 (0.54, 1.54)	1.06 (0.60, 1.89)	1.06 (0.59, 1.90)	0.79	
Multivariable HR (95% CI)[3]	1 [Ref]	1.28 (0.80, 2.03)	0.91 (0.54, 1.54)	1.04 (0.58, 1.85)	1.04 (0.58, 1.87)	0.74	
Extent of mucinous component							
Negative							
Cases, no. ($n = 875$)	142	244	233	124	132		
Age-adjusted HR (95% CI)	1 [Ref]	1.19 (0.97, 1.47)	1.33 (1.07, 1.65)	1.37 (1.07, 1.75)	1.58 (1.24, 2.00)	<0.001	0.36
Multivariable HR (95% CI)[3]	1 [Ref]	1.19 (0.96, 1.47)	1.31 (1.06, 1.63)	1.33 (1.04, 1.71)	1.54 (1.20, 1.97)	<0.001	0.37
1% to 49%							
Cases, no. ($n = 400$)	63	113	104	65	55		
Age-adjusted HR (95% CI)	1 [Ref]	1.18 (0.86, 1.61)	1.32 (0.96, 1.82)	1.51 (1.06, 2.14)	1.38 (0.95, 1.98)	0.03	
Multivariable HR (95% CI)[3]	1 [Ref]	1.17 (0.86, 1.61)	1.30 (0.94, 1.79)	1.47 (1.03, 2.09)	1.34 (0.92, 1.94)	0.06	
≥50%							
Cases, no. ($n = 164$)	21	44	43	20	36		
Age-adjusted HR (95% CI)	1 [Ref]	1.45 (0.86, 2.45)	1.70 (1.00, 2.88)	1.36 (0.73, 2.53)	2.65 (1.54, 4.57)	0.001	
Multivariable HR (95% CI)[3]	1 [Ref]	1.46 (0.86, 2.46)	1.67 (0.98, 2.84)	1.32 (0.71, 2.46)	2.59 (1.50, 4.47)	0.001	

[1]The extent of mucinous components was classified as negative *versus* 1–49% mucinous component *versus* ≥50% mucinous component.
[2]Adjusted for the same set of covariates as in Table 2.
[3]We assessed whether the magnitude of the subtype-specific associations had an increasing or decreasing ordinal trend according to MSI status, tumor differentiation or the extent of mucinous component, using a likelihood ratio test with one degree of freedom and the statistical significance of this trend was presented as $P_{heterogeneity}$.

microenvironment.[36] As inflammation and immunity have vastly complex roles, an accumulation of hypothesis-driven and exploratory studies with different sets of biological markers in multiple populations might provide a better picture of the relationship between adiposity and colorectal carcinogenesis. Hence, this study has explored a novel approach to characterize the host lymphocytic reaction to neoplasm and investigates the role of host immunity in tumor development.

It should be of concern that epidemiological studies assessing biomarkers and subsequent cancer risk might possess several methodologic shortfalls.[37] Given the naturally proinflammatory feature of tumor itself,[38] it is important to evaluate whether the increased inflammatory marker may reflect the causal drives of cancer development or simply enhance the tumor-related inflammation. Thus, prospective large population-based studies might be extremely valuable in addressing the association between inflammation and cancer risk. Furthermore, since inflammation and immune response have been consistently linked to multifaceted host factors including dietary, environmental and lifestyle exposures in subsequent cancer development, a well-designed hypothesis-generating studies might be inevitable to elucidate the causality of inflammation with carcinogenesis. Our current study aims to challenge these traditional methodological caveats[37,39] and provide more comprehensive and resolute data on the lines of the adiposity-related colorectal carcinogenesis applying the molecular pathological epidemiology (MPE) approach.[40–43]

We found no evidence of differential association of BMI with the risk of colorectal cancer according to the level of lymphocytic infiltrates. Our consistent positive association between BMI and each subtype of colorectal carcinoma risk in both women and men could be plausible that BMI might not promote carcinogenesis in a single pathway. Recent intriguing observation for the role of symbiotic gut microbiome demonstrates its underlying complex biological mechanism of body fatness on increased colorectal cancer risk.[44,45] At least in mice, high-fat diet mediated a shift in the composition of intestinal microbiota with host immune suppression and promoted intestinal tumorigenesis regardless of obesity.[44] Further studies should assess the differential susceptibilities for colorectal carcinoma risk by tumor microenvironment and potential influence of host energy metabolism.

Integrative analysis of tumor molecular characteristics and host factors including lifestyle exposure and immune response in the tumor microenvironment is increasingly important in cancer research.[46] Accumulating evidence suggests that the degree of lymphocytic infiltration has been associated with MSI status in colorectal cancer.[10,12] MSI-high neoplastic cells have frameshift mutations, which may elicit a host immune response and improve the patient prognosis. Previous case-control studies have shown that excess body weight was associated with increased risk of MSS colorectal cancers, but not with MSI-high tumors,[13–15] although there has been no definitive evidence to support these findings. In contrast, according to the recent large population-based studies, Hoffmeister *et al.* reported that BMI was significantly associated with increased risk of MSI-high colorectal carcinoma in women,[47] or Hughes *et al.* found no effect modification for the positive association between BMI and the colorectal carcinoma development by MSI status,[48] in agreement with our current data. Additional large-scale population-based studies are warranted to delve into the obesity-colorectal cancer axis according to relevant tumor molecular features and many other types of immune cell infiltrates.

By virtue of its observational design, our study possesses several limitations. First, the possibility of residual confounding by unmeasured factors may not be excluded. Nonetheless, our multivariable risk estimates after adjusted for potential confounders did not substantially differ from the age-adjusted estimates. Second, potential selection bias might arise when we excluded cases without tissue specimen; although the distribution of each risk factor in included cases did not considerably differ from excluded cases. In addition, although some literature has suggested that the role of various immune cells and immunomodulatory factors in the tumor microenvironment as in the lines of cancer development and progression,[49] these findings are still underexamined and lack of sufficient evidence. Since our primary aim was to investigate the role of host immune response and BMI with the risk of colorectal cancer development, we have currently examined the pattern or density of lymphocytic infiltrates in the colorectal tumor specimens, which have been well studied in numerous cancer immunity research.[8–11] Hence, future studies need to assess the relationship between higher BMI and the increased risk of colorectal carcinoma according to the intensities of other type of intratumoral immune cells including myeloid-derived suppressors cells (MDSC), eosinophils, neutrophils or dendritic cells or the expression of immune checkpoint molecules. Finally, the overall sample size and statistical power were limited and thus our results should be interpreted cautiously and validated in independent datasets.

Our current study has several strengths. First, since prospective collection of anthropometric and lifestyle information up to 30 years of follow-up, we could evaluate the long-term effect of BMI, free from the potential for recall bias. The use of cumulative mean weight might likely decrease misclassifications. Second, our detailed, updated data on exposure with a high follow-up rate enabled us to control potential confounders by other dietary and lifestyle factors related to colorectal carcinogenesis. Furthermore, we examined host lymphocytic reaction in the tumor microenvironment and provided a key insight into the complex interaction between exposures, host factors and neoplastic cells, which was unable to assess by utilizing peripheral blood biomarkers. Lastly, our present study utilized a MPE approach,[41,50] which has gained our understanding of the heterogeneous etiology and molecular pathogenesis of colorectal cancer and provided the first line of population-based evidence for patient management.

In conclusion, in our large database of colorectal cancers, higher BMI was associated with increased risk of colorectal carcinoma irrespective of tumor lymphocytic reaction pattern, intensity of T-cell infiltrates or MSI status. Future

studies are required to elucidate the potential role of host immunity on the causal link between adiposity and colorectal cancer development.

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Conflict of Interest

A.T.C. previously served as a consultant for Bayer Healthcare, Millennium Pharmaceuticals, Pozen Inc, and Pfizer Inc. This study was not funded by Bayer Healthcare, Millennium Pharmaceuticals, Pozen Inc or Pfizer Inc. All remaining authors have declared no conflicts of interest.

References

1. Renehan AG, Soerjomataram I, Tyson M, et al.Incident cancer burden attributable to excess body mass index in 30 European countries. *Int J Cancer* 2010;126:692–702.
2. Bhaskaran K, Douglas I, Forbes H, et al. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet* 2014;384:755–65.
3. Kuipers EJ, Grady WM, Lieberman D, et al. Colorectal cancer. *Nat Rev Dis Prim* 2015;15065. [Internet] ;. Available from: http://dx.doi.org/10.1038/nrdp.2015.65
4. Martinez-Useros J, Garcia-Foncillas J. Obesity and colorectal cancer: molecular features of adipose tissue. *J Transl Med* 2016;14:21.
5. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 2012;18:363–74.
6. Booth A, Magnuson A, Fouts J, et al. Adipose tissue, obesity and adipokines: role in cancer promotion. *Horm Mol Biol Clin Investig* 2015;21:57–74.
7. Arthur JC, Perez-Chanona E, Muhlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012;338:120–3.
8. Maby P, Tougeron D, Hamieh M, et al. Correlation between density of CD8+ T cell infiltrates in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res* 2015;75:3446–55.
9. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006;313:1960–4.
10. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. *J Pathol* 2010;222:350–66.
11. Ling A, Edin S, Wikberg ML, et al.The intratumoural subsite and relation of CD8(+) and FOXP3(+) T lymphocytes in colorectal cancer provide important prognostic clues. *Br J Cancer* 2014;110:2551–9.
12. Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the "Immunoscore" in the classification of malignant tumours. *J Pathol* 2014;232:199–209.
13. Slattery ML, Curtin K, Anderson K, et al. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J Natl Cancer Inst* 2000;92:1831–6.
14. Satia JA, Keku T, Galanko JA, et al. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2005;14:429–36.
15. Campbell PT, Jacobs ET, Ulrich CM, et al. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. *J Natl Cancer Inst [Internet]* 2010;102:391–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20208017
16. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009;15:6412–20.
17. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N Engl J Med* 2007;356:2131–42.
18. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med [Internet]* 2012;367:1596–606. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23094721
19. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser [Internet]* 2000;894:i – xii, 1–253. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11234459
20. Rimm EB, Stampfer MJ, Colditz GA, et al.Validity of self-reported waist and hip circumferences in men and women. *Epidemiology* 1990;1:466–73.
21. Ogino S, Odze RD, Kawasaki T, et al. Correlation of pathologic features with CpG island methylator phenotype (CIMP) by quantitative DNA methylation analysis in colorectal carcinoma. *Am J Surg Pathol [Internet]* 2006;30:1175–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16931963
22. Ogino S, Brahmandam M, Cantor M, et al. Distinct molecular features of colorectal carcinoma with signet ring cell component and colorectal carcinoma with mucinous component. *Mod Pathol* 2006;19:59–68.
23. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* 2009;58:90–6.
24. Song M, Nishihara R, Wang M, et al. Plasma 25-hydroxyvitamin D and colorectal cancer risk according to tumour immunity status. *Gut* 2016;65:296–304.
25. Wang M, Spiegelman D, Kuchiba A, et al. Statistical methods for studying disease subtype heterogeneity. *Stat Med* 2016;35:782–800.
26. Kuchiba A, Morikawa T, Yamauchi M, et al. Body mass index and risk of colorectal cancer according to fatty acid synthase expression in the nurses' health study. *J Natl Cancer Inst* 2012;104:415–20.
27. Morikawa T, Kuchiba A, Lochhead P, et al. Prospective analysis of body mass index, physical activity, and colorectal cancer risk associated with beta-catenin (CTNNB1) status. *Cancer Res [Internet]* 2013;73:1600–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23442321
28. Aleman JO, Eusebi LH, Ricciardiello L, et al. Mechanisms of obesity-induced gastrointestinal neoplasia. *Gastroenterology* 2014;146:357–73.
29. Xu XT, Xu Q, Tong JL, et al. Meta-analysis: circulating adiponectin levels and risk of colorectal cancer and adenoma. *J Dig Dis* 2011;12:234–44.
30. Gialamas SP, Sergentanis TN, Antonopoulos CN, et al. Circulating leptin levels and risk of colorectal cancer and adenoma: a case-control study and meta-analysis. *Cancer Causes Control* 2013;24:2129–41.
31. Song M, Zhang X, Wu K, et al. Plasma adiponectin and soluble leptin receptor and risk of colorectal cancer: a prospective study. *Cancer Prev Res (Phila)* 2013;6:875–85.
32. Erlinger TP, Platz EA, Rifai N, et al. C-reactive protein and the risk of incident colorectal cancer. *JAMA* 2004;291:585–90.
33. Aleksandrova K, Jenab M, Boeing H, 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.
34. Chan AT, Ogino S, Giovannucci EL, et al. Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 2011;140:799–808. quiz e11.
35. Song M, Wu K, Ogino S, et al. A prospective study of plasma inflammatory markers and risk of colorectal cancer in men. *Br J Cancer* 2013;108:1891–8.
36. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat Rev Cancer* 2015;15:484–98.
37. Brenner DR, Scherer D, Muir K, et al. A review of the application of inflammatory biomarkers in epidemiologic cancer research. *Cancer Epidemiol Biomarkers Prev* 2014;23:1729–51.
38. Lasry A, Zinger A, Ben-Neriah Y. Inflammatory networks underlying colorectal cancer. *Nat Immunol* 2016;17:230–40.
39. Tsilidis KK, Papatheodorou SI, Evangelou E, et al. Evaluation of excess statistical significance in meta-analyses of 98 biomarker associations with cancer risk. *J Natl Cancer Inst* 2012;104:1867–78.

40. Ogino S, Chan AT, Fuchs CS, et al. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut [Internet]* 2011;60:397–411. Available from: http://gut.bmj.com/content/60/3/397
41. Ogino S, Lochhead P, Chan AT, et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol [Internet]* 2013;26:465–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23307060
42. Nishihara R, VanderWeele TJ, Shibuya K, et al. Molecular pathological epidemiology gives clues to paradoxical findings. *Eur J Epidemiol* 2015;30:1129–35.
43. Ogino S, Campbell PT, Nishihara R, et al. Proceedings of the second international molecular pathological epidemiology (MPE) meeting. *Cancer Causes Control* 2015;26:959–72.
44. Schulz MD, Atay C, Heringer J, et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature* 2014;514:508–12.
45. Hagland HR, Soreide K. Cellular metabolism in colorectal carcinogenesis: influence of lifestyle, gut microbiome and metabolic pathways. *Cancer Lett* 2015;356:273–80.
46. Colussi D, Brandi G, Bazzoli F, et al. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci [Internet]* 2013;14:16365–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23965959
47. Hoffmeister M, Blaker H, Kloor M, et al. Body mass index and microsatellite instability in colorectal cancer: a population-based study. Cancer *Epidemiol Biomarkers Prev* 2013;22:2303–11.
48. Hughes LA, Williamson EJ, van Engeland M, et al. Body size and risk for colorectal cancers showing BRAF mutations or microsatellite instability: a pooled analysis. *Int J Epidemiol [Internet]* 2012;41:1060–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22531127
49. Katoh H, Wang D, Daikoku T, et al. CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis. *Cancer Cell* 2013;24:631–44.
50. Campbell PT, Newton CC, Newcomb PA, et al. Association between body mass index and mortality for colorectal cancer survivors: overall and by tumor molecular phenotype. Cancer *Epidemiol Biomarkers Prev* 2015;24:1229–38.

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