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Body mass index and risk of colorectal carcinoma subtypes classified by tumor differentiation status

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Abstract

Background—Previous studies suggest that abnormal energy balance status may dysregulate intestinal epithelial homeostasis and promote colorectal carcinogenesis, yet little is known about how host energy balance and obesity influence enterocyte differentiation during carcinogenesis. We hypothesized that the association between high body mass index (BMI) and colorectal carcinoma incidence might differ according to tumor histopathologic differentiation status.

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Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols for genes (*italics*) and gene products (non-*italics*), including CTNNB1, FASN, and PPARC; all of which are described at www.genenames.org.

Methods—Using databases of the Nurses' Health Study and Health Professionals Follow-up Study, and duplication-method Cox proportional hazards models, we prospectively examined an association between BMI and the incidence of colorectal carcinoma subtypes classified by differentiation features.

Results—120,813 participants were followed for 26 or 32 years and 1,528 rectal and colon cancer cases with available tumor pathological data were documented. The association between BMI and colorectal cancer risk significantly differed depending on the presence or absence of poorly-differentiated foci ($P_{\text{heterogeneity}}=0.006$). Higher BMI was associated with a higher risk of colorectal carcinoma without poorly-differentiated foci (30.0 kg/m² vs. 18.5–22.4 kg/m²: multivariable-adjusted hazard ratio, 1.87; 95% confidence interval, 1.49–2.34; $P_{\text{trend}} < 0.001$), but not with risk of carcinoma with poorly-differentiated foci ($P_{\text{trend}}=0.56$). This differential association appeared to be consistent in strata of tumor microsatellite instability (MSI) or FASN expression status, although the statistical power was limited. The association between BMI and colorectal carcinoma risk did not significantly differ by overall tumor differentiation, mucinous differentiation, or signet ring cell component ($P_{\text{heterogeneity}} > 0.03$, with the adjusted α of 0.01).

Conclusions—High BMI was associated with risk of colorectal cancer subtype containing no poorly-differentiated focus. Our findings suggest that carcinogenic influence of excess energy balance might be stronger for tumors that retain better intestinal differentiation throughout the tumor areas.

Keywords

epigenetics; glandular epithelium; metabolism; molecular pathological epidemiology; overweight; stem cell

Introduction

Obesity represents a growing global concern in public health.[1–4] Although epidemiologic studies have shown an association between obesity or high body mass index (BMI) with increased colorectal cancer incidence[5,6] and mortality,[7,8] the underlying pathogenic mechanisms remain unclear.

Accumulating evidence suggests that excess energy balance may not only influence enterocyte differentiation but also contribute to colorectal carcinogenesis.[9–15] Experimental studies suggest that excess fat intake may increase both the number of intestinal stem cells, which may serve as the cell-of-origin for the development of cancer, and the proliferation rate of these intestinal stem cells.[14,16] Excess fat intake may also enhance the ability of more differentiated enterocytes (“transit-amplifying cells”), which are derived from intestinal stem cells, to initiate tumor development.[14,15] This effect appears to be mediated by PPAR δ activation in colorectal epithelial cells.[14,16] This activation may induce colonic inflammation and inflammation-associated tumorigenesis,[16] although the underlying mechanism of tumorigenesis has not been fully elucidated. Colorectal carcinoma is a tumor type with highly heterogeneous histopathological and molecular characteristics,[17–19] and previous studies suggest that excess energy balance likely affects enterocyte differentiation.[9–15] Thus, comprehensive analyses that take tumor

histopathological variation into account are critical to elucidate the pathogenic influence of obesity on colorectal cancer development.

Poorly-differentiated colorectal carcinoma, characterized by 50% or greater poorly-differentiated areas, has been associated with distinct morphological and molecular characteristics including high level microsatellite instability (MSI), FASN overexpression, and the presence of mucinous or signet ring cell components.[20–22] MSI-high colorectal cancer has a deficient DNA mismatch repair system[23] and has been associated with poorly-differentiated tumors,[21] although the underlying mechanism remains unclear. FASN, a key enzyme involved in *de novo* lipogenesis, is commonly overexpressed in human tumors,[24,25] including colorectal carcinoma.[20,26] FASN overexpression has been associated with an increased risk of metastatic disease and worse prognosis.[27] Notably, studies using recently developed FASN inhibitors provide evidence that FASN downregulation can cause cell apoptosis and may suppress tumor proliferation in certain cancer types.[28,29]

Consistent with this accumulating evidence for the tumor-promoting effects of obesity, several studies have shown an association between higher BMI and the incidence of colorectal carcinoma, especially those with microsatellite stable (MSS) subtype (but not MSI-high subtype).[30–32] Nonetheless, other studies do not support this differential association.[33–35] Our previous study has shown an association between BMI and the incidence of colorectal carcinoma without FASN overexpression (but not FASN-overexpressed carcinoma).[36] Although there may be a relationship between BMI and the incidence of specific histopathological subtypes of colorectal carcinoma, no study has investigated the influence of excess energy balance on the risk of colorectal cancer according to tumor morphological features. Given the possible association between obesity and MSS colorectal carcinoma,[30–32] or FASN-negative carcinoma,[36] which are rarely associated with poorly-differentiated tumors,[20,21] we hypothesized that higher BMI might be associated with a greater incidence of well-differentiated colorectal carcinoma or colorectal carcinoma with no poorly-differentiated foci.

A prospective study was conducted using a molecular pathological epidemiology[37–40] database based on two U.S.-nationwide prospective cohort studies. In secondary analyses, we assessed the hypothesis in strata of relevant molecular phenotypes of colorectal cancer, including MSI and FASN expression status.

Methods

Study population

We utilized two large prospective cohort studies in U.S.; the Nurses' Health Study (NHS, 121,700 female nurses aged 30–55 were enrolled in 1976) and the Health Professionals Follow-up Study (HPFS, 51,529 male health professionals aged 40–75 were enrolled in 1986).[41,42] The cohorts have mailed questionnaires at enrollment and every two years thereafter to collect data on demographics, lifestyle factors, medical history, and disease outcomes. Information on dietary intake was collected every four years. Written informed consent was obtained from all individuals. The institutional review boards at the Harvard

T.H. Chan School of Public Health and Partners' Healthcare approved this study. We excluded participants who had a history of cancer (except for non-melanoma skin cancer) or inflammatory bowel disease, or those with incomplete data on weight or height at baseline. We also excluded participants with a baseline BMI less than 18.5 kg/m² since these individuals might be underweight due to their preclinical disease or unhealthy condition. Participants were followed from the month of the baseline questionnaire return to the month of colorectal cancer diagnosis, death, or censoring date (June 1, 2010 for the NHS and January 31, 2010 for the HPFS), whichever came first. We treated colorectal carcinoma cases without tumor tissue data as censored cases at the month of the diagnosis.

Assessment of weight and height

To calculate average of BMI from study baseline to the time period of risk, we used height information reported at enrollment (1976 for the NHS and 1986 for the HPFS) and the average weight information up to time period at risk, using weight information collected every two years. In both cohorts, the validity and the reproducibility of the self-reported weight information was reasonable as previously described.[43] Participants were followed by trained technicians twice during the study, approximately 6 months apart to incorporate seasonal variability, and measured current their weight. Pearson correlation coefficient between self-reported weight and the average of the two weights measured by the technicians was 0.97 for both women and men.[43] Based on the World Health Organization (WHO) classification, we categorized BMI (kg/m²) into 5 categories: 18.5-22.4, 22.5-24.9, 25.0-27.4, 27.5-29.9, 30.0 kg/m². [44] To capture the change of BMI in each individual during the follow-up period, we have performed a Pearson correlation between the baseline BMI and the BMI of each biennial questionnaire cycle in each cohort. The Pearson correlation coefficients for BMI one decade apart was about 0.85, and for two decades apart, it was about 0.75, suggesting that the participants' weights were fairly constant over time (data were not shown).

Assessment of colorectal cancer cases

We requested written permission to obtain medical records and pathology reports when participants reported colorectal cancer on biennial questionnaires. Unreported fatal colorectal cases were identified through the National Death Index and next-of-kin. Medical and pathological records were reviewed by study physicians, who were blinded to exposure information, to collect data on colorectal cancer including tumor anatomic location, and disease stage.

Tumor histopathological, immunohistochemical, and molecular evaluation

We collected formalin-fixed paraffin-embedded (FFPE) archival tissue specimens of colorectal carcinoma resections from hospitals and laboratories. Hematoxylin and eosin-stained tissue sections from all colorectal carcinoma cases in this study were evaluated under a light microscope by a single pathologist (S.O.) who was unaware of other data. Overall tumor differentiation was categorized as well/moderate vs. poor (50% vs. <50% glandular area, respectively) according to the WHO classification system.[45] A poorly-differentiated focus was defined as an area with (1) solid tumor cell nest without glandular differentiation, or (2) any number of signet ring cells. The extent of each feature of poorly-differentiated

foci, mucinous component, and signet ring cell component was scaled as 0% to 100%. For FASN immunohistochemistry assay, mouse monoclonal antihuman FASN antibody (clone 23, BD Biosciences, Mississauga, ON, Canada) (dilution 1:100) was used as primary antibody, following with Multilink secondary antibody (BioGenex) and streptavidin horseradish peroxidase (BioGenex), as described previously.[36] Tumor expression levels were compared with normal colonic mucosa as an internal negative control, and adipose tissue as an internal positive control. In addition, we included a negative control reaction omitting the primary antibody. Tumor FASN expression levels were categorized as negative vs. positive (no or weak expression vs. moderate or strong expression, respectively) as previously described.[36] DNA was extracted from archival tumor tissue. MSI analysis was performed utilizing 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) and polymerase chain reaction as previously described.[46] We defined MSI-high as the presence of instability in 30% of these markers, and MSI-low/MSS otherwise.

Statistical analysis

We utilized a duplication-method Cox proportional cause-specific hazards regression model to examine whether the association of BMI with risk of colorectal carcinoma cases by tumor subgroups based on histopathologic features (poorly-differentiated foci, overall tumor differentiation, mucinous differentiation, or signet ring cell component).[47] To analyze the association specific to one tumor subtype, we have treated other subgroups as competing events, and tumors of unknown subgroup (such as those without histopathological data) were censored as previously described.[48] Age-adjusted and multivariable-adjusted hazard ratio (HR) was computed. In multivariable models, we adjusted for the following potential confounders: family history of colorectal cancer in any first-degree relative (yes vs. no), history of colonoscopy or sigmoidoscopy (yes vs. no), smoking status (never, <5, 5-19, 20-39, 40 cumulative pack-years), physical activity (quintiles of metabolic equivalent task score [METs]-hours per week), red and processed meat intake (quintiles of servings per day), alcohol consumption (0, 1-4, 5-14, 15-29, 30 gram per day), current multivitamin use (yes vs. no), regular use of aspirin (yes vs. no), regular use of non-steroidal anti-inflammatory drugs (NSAIDs, yes vs. no), total energy intake (quintiles of kcal per day), folate intake (quintiles of µg per day), calcium intake (quintiles of mg per day), Alternate Healthy Eating Index (AHEI)-2010 (quintiles of the overall AHEI-2010 score without alcohol intake) and (for women participants) menopause/postmenopausal hormone-replacement therapy use status (premenopausal, postmenopausal never hormone use, postmenopausal past hormone use, or postmenopausal current hormone use). We used the most recent available information for all covariates prior to each biennial questionnaire cycle. The cumulative average of each relevant variable, which was the mean of all available data up to each biennial follow-up cycle, was used to decrease within-individual variation and to optimally estimate long-term influence. For missing variables, we used the most recent available information from the past questionnaires. We treated all variables as time-varying to take into account potential changes over follow-up time. Trend tests across categories of BMI were performed by using the median value for each category as a continuous variable in the regression model. We initially performed the analyses in each cohort separately and did not detect substantial heterogeneity between cohorts

($P_{\text{heterogeneity}} > 0.05$ for Cochran's Q test); thus, we conducted a pooled analysis using the sex-stratified Cox regression model to maximize statistical power.

As our primary hypothesis testing, we examined the heterogeneity in the association between BMI and the colorectal cancer incidence according to tumor histologic subtypes using a likelihood ratio test with one degree of freedom (represented as $P_{\text{heterogeneity}}$). [47,49] We evaluated four morphological features (overall tumor differentiation, the presence of poorly-differentiated foci, or mucinous component, and signet ring cells) and therefore, adjusted the statistical significance level to 0.01 ($\approx 0.05/4$) based on Bonferroni correction for multiple hypothesis testing. All other assessments were secondary analyses. For secondary analyses, we interpreted the results cautiously in addition to use of the adjusted α level of 0.01. For example, as secondary analyses, we stratified cases by MSI or FASN status and examined the association between BMI and colorectal carcinoma risk according to histological subtypes after stratification by MSI or FASN status. All analyses were performed using SAS software (Version 9.3, SAS Institute, Cary, NC, USA) and all P values were two-sided.

Results

Among 120,813 participants (75,812 women and 45,001 men) during the 32 or 26 years of follow-up (3,346,671 person-years), we documented 1,528 incident colorectal carcinoma cases with and 1,446 cases without available FFPE tumor tissue and histopathologic records. Demographic characteristics and clinical features were similar between patients with and without tissue data, except that stage IV cancers were relatively under-represented in those with tissue data (Table S1). In both cohorts, individuals with higher BMI were more likely to have a history of diabetes, regularly use of NSAIDs or aspirin, consume more alcohol and red and processed meat, exercise less, and take more multivitamins, calcium, and folate (Table 1). Postmenopausal women with lower BMI tended to use menopausal hormone therapy, compared with those with higher BMI. The interrelationship between the subtypes based on each of the four morphological features (overall tumor differentiation, the presence of poorly-differentiated foci, or mucinous component, and signet ring cells) were shown in Table S2. In agreement with our previous study, [20] colorectal carcinoma with poor differentiation or those with the presence of poorly-differentiated focus were associated with tumors with high abundance of mucinous or signet ring cell components (Table S2).

Higher BMI was significantly associated with a higher risk of overall colorectal cancer for both women and men ($P_{\text{trend}} < 0.001$) (Tables 2 and 3). In our primary hypothesis testing, the association of BMI with risk of colorectal cancer differed significantly by presence vs. absence of poorly-differentiated foci ($P_{\text{heterogeneity}} = 0.006$) (Table 4). The association between BMI and a higher risk of colorectal cancer appeared to be specific for cancer without poorly-differentiated foci (comparing participants with 30.0 kg/m^2 to those with $18.5\text{--}22.4 \text{ kg/m}^2$; multivariable-adjusted HR=1.87; 95% CI, 1.49–2.34; $P_{\text{trend}} < 0.001$). In contrast, BMI was not significantly associated with incidence of colorectal cancer with poorly-differentiated foci (comparing participants with 30.0 kg/m^2 to those with $18.5\text{--}22.4 \text{ kg/m}^2$; multivariable-adjusted HR=1.17; 95% CI, 0.82–1.65; $P_{\text{trend}} > 0.50$) (Table 4). Although the heterogeneity test did not reach statistical significance with Bonferroni

correction, similar heterogeneity was observed in analyses of colorectal cancer subtypes classified by overall tumor differentiation ($P_{\text{heterogeneity}}=0.04$, with the adjusted α level of 0.01) (Table 4). These differential associations appeared consistent in both cohorts (Tables 2 and 3). On the contrary, we did not observe differential association of BMI with colorectal cancer incidence by subtypes classified by mucinous or signet ring cell components ($P_{\text{heterogeneity}}>0.03$) (Tables 4 and S5).

In agreement with our previous studies,[35] there was no statistically significant heterogeneity in the associations between BMI and cancer subtypes according to MSI ($P_{\text{heterogeneity}}=0.72$) (Table S3). However, we observed a significant heterogeneity in the association of BMI with cancer subtypes by FASN expression status ($P_{\text{heterogeneity}}=0.008$) (Table S4). Because of the associations of MSI-high colorectal cancer with poor differentiation[21] and FASN overexpression,[22] as well as the association of obesity with FASN-negative colorectal cancer risk,[36] we further investigated whether the heterogeneous association according to the presence of poorly-differentiated foci was independent of MSI or FASN status. Although the statistical power was limited in the subsequent subgroup analyses, the differential association of BMI with colorectal cancer subtypes according to the presence of poorly-differentiated foci appeared to be consistent regardless of MSI or FASN expression status (Tables S3 and S4).

Discussion

A large prospective cohort study was conducted to assess the association between BMI and colorectal cancer risk according to subtypes defined by tumor histomorphological features. Accumulating evidence suggests that poor differentiation or the presence of poorly-differentiated focus is associated with colorectal carcinoma with MSI-high,[21] FASN overexpression,[22] and mucinous or signet ring cell components.[20] MSI-high, characterized by a deficient DNA mismatch repair system,[23] and overexpression of FASN, a key enzyme of *de novo* lipogenesis,[20,26] appeared to drive dysregulated cell proliferation, reducing gland-forming ability of neoplastic epithelial cells, independent of excess energy balance. Colorectal carcinoma with a high abundance of mucinous or signet ring cell components could potentially be poorly differentiated tumors or those with the presence of poorly-differentiated focus,[45] consistent with our previous study.[20] Collectively, obesity may influence colorectal carcinoma risk differentially according to tumor morphological characteristics. The current study suggests that higher BMI may be associated with an increased risk of colorectal cancer without poorly-differentiated foci. This heterogeneous association appeared to be present irrespective of MSI or FASN expression status, although the statistical power was limited. We also observed a possibly stronger association between BMI and well/moderately differentiated colorectal carcinoma as compared with the association between BMI and poor differentiation carcinoma, although the difference was not statistically significant. Our data suggest that excess body weight might contribute to the development of specific morphologic subtypes of colorectal cancer.

Accumulating evidence from epidemiological and animal studies suggests a causal link between obesity and colorectal cancer,[50–52] but little is known about how excess energy intake can drive carcinogenesis in the intestinal epithelium.[15,53–55] In a recent large

population-based study of U.K. women, BMI was analyzed in relation to risks of mucinous and signet ring cell subtypes of colorectal carcinoma, but poor differentiation or poorly differentiated focus was not examined.[56] Notably, the classification system of neuroendocrine type vs. signet ring cell type vs. mucinous type (vs. other adenocarcinoma) is not optimal, because two or three of these features can co-exist in a given tumor. Moreover, their histopathological data were not based on centralized pathology review, but largely on pathological reports in community hospital settings.[56] In the current study, pathologic characteristics were examined by the single expert pathologist, and our multivariable analyses adjusting for potential confounders could demonstrate a vigorous heterogeneous association between BMI and colorectal cancer incidence according to tumor differentiation.

Previous studies have shown that excess energy balance and the resultant PPARD activation may play a critical role in tissue remodeling and cancer initiation in the intestine. [9,13,14,57–59] Recent studies in murine models have demonstrated that prolonged high-fat load can increase the proportion of intestinal stem cells (ISCs), augment stem cell self-renewal, and provide non-ISCs (differentiated intestinal cells) with stemness attributes through activation of PPARD signaling, all of which may contribute to tumor development. [14,15] Additional studies have shown that the number of ISCs may be reduced by fasting and increased by refeeding.[10] In contrast, one study has shown that prolonged energy restriction leads to an increase in ISC proliferation, and an enhancement of ISC activity in mice.[12] The present study provides evidence suggesting that higher BMI is associated with an increased risk of colorectal carcinoma that has an abundance of well-differentiated glandular epithelial cells (i.e., no poorly-differentiated focus). The data in the current study suggest that colorectal carcinoma containing fewer malfunctioning stem-like cells (which may fail to differentiate into glandular epithelial cells) may depend on excess energy balance for their growth, while carcinoma containing many such malfunctioning stem-like cells may be less dependent on systemic energy balance status. This hypothesis requires further investigation in future studies.

The results of epidemiologic studies are somewhat conflicting in terms of the association of BMI with incidence of colorectal cancer MSI-high and MSS subtypes. Although earlier case-control studies suggested the differential influence of obesity on MSS vs. MSI-high colorectal cancer risk,[30–32] subsequent prospective cohort studies did not fully support this differential association.[33–35] We can speculate that the previously-described association between BMI and MSS colorectal cancer might have been driven by the association between MSS and carcinoma without poorly-differentiated foci. Another possible explanation for the association between obesity and MSS colorectal cancer might be due to the higher prevalence of MSS status in FASN-negative colorectal cancer.[22,40] As we previously demonstrated, higher BMI might be associated with incidence of FASN-negative colorectal cancer, but not with FASN-positive cancer.[36] Together with experimental for the “metabolic oncogenic” role of tumor FASN,[26,60,61] it is conceivable that FASN-overexpressing colonic cells might initiate tumorigenesis independent of host energy balance status whereas FASN-negative cells might depend on excess energy balance for neoplastic development. Indeed in our exploratory analysis, FASN-negative colorectal

carcinoma without poorly-differentiated foci appeared to be most strongly associated with high BMI.

As the emerging field of epidemiology, molecular pathological epidemiology (MPE) facilitates integrative analyses on the associations of lifestyle, dietary or environmental factors with cancer incidence and mortality, taking into account tumor genetic, epigenetic, or histopathological variations.[62] The MPE approach[63,64] can take into account histopathologic characteristics as manifestations of molecular pathology. The current study exploiting this MPE approach can provide a unique insight into mechanisms of colorectal carcinogenesis associated with excess adiposity. Additionally, there are several strengths in this study. First, we avoided recall bias in weight reporting by using a prospective study design. Second, repeated collection of prediagnosis weight data over a prolonged follow-up period allowed us to evaluate the long-term effect of adiposity on colorectal cancer risk. To control for potential confounders, we adjusted for a number of dietary and environmental factors. In addition, tissue specimens of colorectal cancer were collected from a large number of hospitals in various settings throughout the U.S., which increases the generalizability of our findings.

There are several limitations in this study. First, tumor tissue specimens were not available for all colorectal cancer cases, leading to potential selection bias. However, we did not observe any considerable differences in demographic features between participants with and without available tissue data. In addition, we acknowledge possible bias due to intratumor heterogeneity in evaluating tumor histopathologic characteristics. Given the fact that we examined focal features of each tumor specimen, including poorly-differentiated focus, mucinous component, and signet ring cells, intratumor heterogeneity was indeed taken into account in our analyses. Second, as is the case with observational studies, there is the possibility of unmeasured confounding. Nonetheless, our multivariable risk estimates were computed with adjustment for many well-known risk factors for colorectal cancer. Third, the current data were derived from cohort studies of health care professionals who were mostly Caucasians. Hence, generalizability of our findings in other populations must be examined in additional studies. Fourth, there has been a debate on use of BMI as a surrogate for host energy balance status.[65] Therefore, future studies using different anthropometric factors would provide additional insights into excess energy intake and colorectal carcinogenesis. Fifth, because of the MPE research design, this study possesses specific caveats, which have been previously discussed.[37,38] In particular, recognizing multiple hypothesis testing in our current analyses, we used the adjusted α level, and interpreted our results cautiously. In addition, we emphasize the importance of validating our findings in independent population-based studies in the future.

In conclusion, our MPE study suggests that higher BMI might increase the risk of colorectal carcinoma without poorly-differentiated foci. Although a validation in independent datasets is needed, our findings may provide unique insights into the potential oncogenic role of obesity and excess energy balance status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Renehan AG, Soerjomataram I, Tyson M, Egger M, Zwahlen M, Coebergh JW, Buchan I. Incident cancer burden attributable to excess body mass index in 30 European countries. *Int J Cancer*. 2010; 126(3):692–702. [PubMed: 19645011]
2. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet*. 2014; 384(9945):755–65. [PubMed: 25129328]
3. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJH, Watanabe T. Colorectal cancer. *Nat Rev Dis Prim*. 2015; 15065
4. Martinez-Useros J, Garcia-Foncillas J. Obesity and colorectal cancer: molecular features of adipose tissue. *J Transl Med*. 2016; 14(1):21. [PubMed: 26801617]
5. Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. *PLoS One*. 2013; 8(1):e53916. [PubMed: 23349764]
6. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. *Horm Mol Biol Clin Investig*. 2015; 21(1):57–74.
7. Fedirko V, Romieu I, Aleksandrova K, Pischon T, Trichopoulos D, Peeters PH, Romaguera-Bosch D, Bueno-de-Mesquita HB, Dahm CC, Overvad K, Chirlaque MD, Johansen C, Bidstrup PE, Dalton SO, Gunter MJ, Wark PA, Norat T, Halkjaer J, Tjonneland A, Dik VK, Siersema PD, Boutron-Ruault MC, Dossus L, Bastide N, Kuhn T, Kaaks R, Boeing H, Trichopoulou A, Klinaki E, Katsoulis M, Pala V, Panico S, Tumino R, Palli D, Vineis P, Weiderpass E, Skeie G, Gonzalez CA, Sanchez MJ, Barricarte A, Amiano P, Quiros JR, Manjer J, Jirstrom K, Ljuslinder I, Palmqvist R, Khaw KT, Wareham N, Bradbury KE, Stepien M, Duarte-Salles T, Riboli E, Jenab M. Pre-diagnostic anthropometry and survival after colorectal cancer diagnosis in Western European populations. *Int J Cancer*. 2014; 135(8):1949–60. [PubMed: 24623514]
8. Campbell PT, Newton CC, Newcomb PA, Phipps AI, Ahnen DJ, Baron JA, Buchanan DD, Casey G, Cleary SP, Cotterchio M, Farris AB, Figueiredo JC, Gallinger S, Green RC, Haile RW, Hopper JL, Jenkins MA, Le Marchand L, Makar KW, McLaughlin JR, Potter JD, Renehan AG, Sinicrope FA, Thibodeau SN, Ulrich CM, Win AK, Lindor NM, Limburg PJ. Association between body mass index and mortality for colorectal cancer survivors: overall and by tumor molecular phenotype. *Cancer Epidemiol Biomarkers Prev*. 2015; 24(8):1229–38. [PubMed: 26038390]
9. McLeod CJ, Wang L, Wong C, Jones DL. Stem cell dynamics in response to nutrient availability. *Curr Biol*. 2010; 20(23):2100–5. [PubMed: 21055942]

10. O'Brien LE, Soliman SS, Li X, Bilder D. Altered modes of stem cell division drive adaptive intestinal growth. *Cell*. 2011; 147(3):603–14. [PubMed: 22036568]
11. Stelzner M, Helmrath M, Dunn JC, Henning SJ, Houchen CW, Kuo C, Lynch J, Li L, Magness ST, Martin MG, Wong MH, Yu J. A nomenclature for intestinal in vitro cultures. *Am J Physiol Gastrointest Liver Physiol*. 2012; 302(12):G1359–63. [PubMed: 22461030]
12. Yilmaz OH, Katajisto P, Lamming DW, Gultekin Y, Bauer-Rowe KE, Sengupta S, Birsoy K, Dursun A, Yilmaz VO, Selig M, Nielsen GP, Mino-Kenudson M, Zukerberg LR, Bhan AK, Deshpande V, Sabatini DM. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature*. 2012; 486(7404):490–5. [PubMed: 22722868]
13. Mah AT, Van Landeghem L, Gavin HE, Magness ST, Lund PK. Impact of diet-induced obesity on intestinal stem cells: hyperproliferation but impaired intrinsic function that requires insulin/IGF1. *Endocrinology*. 2014; 155(9):3302–14. [PubMed: 24914941]
14. Beyaz S, Mana MD, Roper J, Kedrin D, Saadatpour A, Hong SJ, Bauer-Rowe KE, Xifaras ME, Akkad A, Arias E, Pinello L, Katz Y, Shinagare S, Abu-Remaileh M, Mihaylova MM, Lamming DW, Dogum R, Guo G, Bell GW, Selig M, Nielsen GP, Gupta N, Ferrone CR, Deshpande V, Yuan GC, Orkin SH, Sabatini DM, Yilmaz OH. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature*. 2016; 531(7592):53–8. [PubMed: 26935695]
15. Blomain ES, Waldman SA. Does obesity promote the development of colorectal cancer? *Expert Rev Anticancer Ther*. 2016; 16(5):465–7. [PubMed: 26943700]
16. Wang D, Fu L, Ning W, Guo L, Sun X, Dey SK, Chaturvedi R, Wilson KT, DuBois RN. Peroxisome proliferator-activated receptor δ promotes colonic inflammation and tumor growth. *Proc Natl Acad Sci U S A*. 2014; 111(19):7084–9. [PubMed: 24763687]
17. Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci*. 2013; 14(8):16365–85. [PubMed: 23965959]
18. Kocarnik JM, Shiovitz S, Phipps AI. Molecular phenotypes of colorectal cancer and potential clinical applications. *Gastroenterol Rep (Oxf)*. 2015; 3(4):269–76. [PubMed: 26337942]
19. Kudryavtseva AV, Lipatova AV, Zaretsky AR, Snezhkina AV, Kaprin AD, Alekseev BY. Important molecular genetic markers of colorectal cancer. 7(33)
20. Ogino S, Brahmandam M, Cantor M, Namgyal C, Kawasaki T, Kirkner G, Meyerhardt JA, Loda M, Fuchs CS. Distinct molecular features of colorectal carcinoma with signet ring cell component and colorectal carcinoma with mucinous component. *Mod Pathol*. 2006; 19(1):59–68. [PubMed: 16118624]
21. Ogino S, Odze RD, Kawasaki T, Brahmandam M, Kirkner GJ, Laird PW, Loda M, Fuchs CS. Correlation of pathologic features with CpG island methylator phenotype (CIMP) by quantitative DNA methylation analysis in colorectal carcinoma. *Am J Surg Pathol*. 2006; 30(9):1175–83. [PubMed: 16931963]
22. Ogino S, Kawasaki T, Ogawa A, Kirkner GJ, Loda M, Fuchs CS. Fatty acid synthase overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. *Hum Pathol*. 2007; 38(6):842–9. [PubMed: 17350669]
23. Haydon AMM, Jass JR. Emerging pathways in colorectal-cancer development. *Lancet Oncol*. 2002; 3(2):83–8. [PubMed: 11905459]
24. Rossi S, Graner E, Febbo P, Weinstein L, Bhattacharya N, Onody T, Bubley G, Balk S, Loda M. Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. *Mol Cancer Res*. 2003; 1(10):707–15. [PubMed: 12939396]
25. Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res*. 2006; 66(12):5977–80. [PubMed: 16778164]
26. Rashid A, Pizer ES, Moga M, Milgram LZ, Zahurak M, Pasternack GR, Kuhajda FP, Hamilton SR. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. *Am J Pathol*. 1997; 150(1):201–8. [PubMed: 9006336]
27. Luque-Garcia JL, Martinez-Torrecuadrada JL, Epifano C, Canamero M, Babel I, Casal JJ. Differential protein expression on the cell surface of colorectal cancer cells associated to tumor metastasis. *Proteomics*. 2010; 10(5):940–52. [PubMed: 20049862]

28. Carvalho MA, Zecchin KG, Seguin F, Bastos DC, Agostini M, Rangel AL, Veiga SS, Raposo HF, Oliveira HC, Loda M, Coletta RD, Graner E. Fatty acid synthase inhibition with Orlistat promotes apoptosis and reduces cell growth and lymph node metastasis in a mouse melanoma model. *Int J Cancer*. 2008; 123(11):2557–65. [PubMed: 18770866]
29. Murata S, Yanagisawa K, Fukunaga K, Oda T, Kobayashi A, Sasaki R, Ohkohchi N. Fatty acid synthase inhibitor cerulenin suppresses liver metastasis of colon cancer in mice. *Cancer Sci*. 2010; 101(8):1861–5. [PubMed: 20491775]
30. Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, Schaffer D, Potter J, Leppert M, Samowitz WS. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J Natl Cancer Inst*. 2000; 92(22):1831–6. [PubMed: 11078760]
31. Satia JA, Keku T, Galanko JA, Martin C, Doctolero RT, Tajima A, Sandler RS, Carethers JM. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(2):429–36. [PubMed: 15734969]
32. Campbell PT, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, Haile RW, Jacobs EJ, Newcomb PA, Potter JD, Le Marchand L, Green RC, Parfrey P, Younghusband HB, Cotterchio M, Gallinger S, Jenkins MA, Hopper JL, Baron JA, Thibodeau SN, Lindor NM, Limburg PJ, Martinez ME. Colon Cancer Family R. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. *J Natl Cancer Inst*. 2010; 102(6):391–400. [PubMed: 20208017]
33. Hughes LA, Williamson EJ, van Engeland M, Jenkins MA, Giles GG, Hopper JL, Southey MC, Young JP, Buchanan DD, Walsh MD, van den Brandt PA, Alexandra Goldbohm R, Weijenberg MP, English DR. Body size and risk for colorectal cancers showing BRAF mutations or microsatellite instability: a pooled analysis. *Int J Epidemiol*. 2012; 41(4):1060–72. [PubMed: 22531127]
34. Hoffmeister M, Blaker H, Kloor M, Roth W, Toth C, Herpel E, Frank B, Schirmacher P, Chang-Claude J, Brenner H. Body mass index and microsatellite instability in colorectal cancer: a population-based study. *Cancer Epidemiol Biomarkers Prev*. 2013; 22(12):2303–11. [PubMed: 24127414]
35. Hanyuda A, Ogino S, Rong Qian Z, Nishihara R, Song M, Mima K, Inamura K, Masugi Y, Wu K, Meyerhardt JA, Chan AT, Fuchs CS, Giovannucci EL, Cao Y. Body mass index and risk of colorectal cancer according to tumor lymphocytic infiltrate. *Int J Cancer*. 2016; 139:854–68. [PubMed: 27037951]
36. Kuchiba A, Morikawa T, Yamauchi M, Imamura Y, Liao X, Chan AT, Meyerhardt JA, Giovannucci E, Fuchs CS, Ogino S. 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(5):415–20. [PubMed: 22312135]
37. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut*. 2011; 60(3):397–411. [PubMed: 21036793]
38. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, Meyerhardt JA, Meissner A, Schernhammer ES, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol*. 2013; 26(4):465–84. [PubMed: 23307060]
39. Ogino S, Campbell PT, Nishihara R, Phipps AI, Beck AH, Sherman ME, Chan AT, Troester MA, Bass AJ, Fitzgerald KC, Irizarry RA, Kelsey KT, Nan H, Peters U, Poole EM, Qian ZR, Tamimi RM, Tchetgen Tchetgen EJ, Tworoger SS, Zhang X, Giovannucci EL, van den Brandt PA, Rosner BA, Wang M, Chatterjee N, Begg CB. Proceedings of the second international molecular pathological epidemiology (MPE) meeting. *Cancer Causes Control*. 2015; 26(7):959–72. [PubMed: 25956270]
40. Hamada T, Keum N, Nishihara R, Ogino S. Molecular pathological epidemiology: new developing frontiers of big data science to study etiologies and pathogenesis. *J Gastroenterol*. 2017; 52(3):265–75. [PubMed: 27738762]
41. Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B, Stampfer MJ. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet*. 1991; 338(8765):464–8. [PubMed: 1678444]

42. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Women's Heal.* 1997; 6(1):49–62.
43. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology.* 1990; 1(6):466–73. [PubMed: 2090285]
44. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser.* 2000; 894:i–xii. 1–253. [PubMed: 11234459]
45. World Health Organization Classification of Tumours of the Digestive System. Vol. 2010. Lyon, France: International Agency for Research on Cancer (IARC); p. 134–46.
46. Ogino S, Noshio K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut.* 2009; 58(1):90–6. [PubMed: 18832519]
47. Wang M, Spiegelman D, Kuchiba A, Lochhead P, Kim S, Chan AT, Poole EM, Tamimi R, Tworoger SS, Giovannucci E, Rosner B, Ogino S. Statistical methods for studying disease subtype heterogeneity. *Stat Med.* 2016; 35(5):782–800. [PubMed: 26619806]
48. Cao Y, Nishihara R, Qian ZR, Song M, Mima K, Inamura K, Nowak JA, Drew DA, Lochhead P, Noshio K, Morikawa T, Zhang X, Wu K, Wang M, Garrett WS, Giovannucci EL, Fuchs CS, Chan AT, Ogino S. Regular Aspirin Use Associates With Lower Risk of Colorectal Cancers With Low Numbers of Tumor-Infiltrating Lymphocytes. *Gastroenterology.* 2016; 151(5):879–92.e4. [PubMed: 27475305]
49. Song M, Nishihara R, Wang M, Chan AT, Qian ZR, Inamura K, Zhang X, Ng K, Kim SA, Mima K, Sukawa Y, Noshio K, Fuchs CS, Giovannucci EL, Wu K, Ogino S. Plasma 25-hydroxyvitamin D and colorectal cancer risk according to tumour immunity status. *Gut.* 2016; 65(2):296–304. [PubMed: 25591978]
50. Aleman JO, Eusebi LH, Ricciardiello L, Patidar K, Sanyal AJ, Holt PR. Mechanisms of obesity-induced gastrointestinal neoplasia. *Gastroenterology.* 2014; 146(2):357–73. [PubMed: 24315827]
51. Hagland HR, Soreide K. Cellular metabolism in colorectal carcinogenesis: Influence of lifestyle, gut microbiome and metabolic pathways. *Cancer Lett.* 2015; 356(2 Pt A):273–80. [PubMed: 24614287]
52. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat Rev Cancer.* 2015; 15(8):484–98. [PubMed: 26205341]
53. Hughes LA, Simons CC, van den Brandt PA, Goldbohm RA, de Goeij AF, de Bruine AP, van Engeland M, Weijenberg MP. Body size, physical activity and risk of colorectal cancer with or without the CpG island methylator phenotype (CIMP). *PLoS One.* 2011; 6(4):e18571. [PubMed: 21483668]
54. Wang D, Dubois RN. Associations between obesity and cancer: the role of fatty acid synthase. *J Natl Cancer Inst.* 2012; 104(5):343–5. [PubMed: 22312133]
55. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology.* 2015; 148(6):1244–60.e16. [PubMed: 25575572]
56. Buron Pust A, Alison R, Blanks R, Pirie K, Gaitskell K, Barnes I, Gathani T, Reeves G, Beral V, Green J. Heterogeneity of colorectal cancer risk by tumour characteristics: Large prospective study of UK women. *Int J Cancer.* 2017; 140(5):1082–90. [PubMed: 27859268]
57. Mihaylova MM, Sabatini DM, Yilmaz OH. Dietary and metabolic control of stem cell function in physiology and cancer. *Cell Stem Cell.* 2014; 14(3):292–305. [PubMed: 24607404]
58. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science (80-).* 2015; 347(6217):78–81.
59. Wu S, Powers S, Zhu W, Hannun YA. Substantial contribution of extrinsic risk factors to cancer development. *Nature.* 2016; 529(7584):43–7. [PubMed: 26675728]
60. Uddin S, Hussain AR, Ahmed M, Abubaker J, Al-Sanea N, Abduljabbar A, Ashari LH, Alhomoud S, Al-Dayel F, Bavi P, Al-Kuraya KS. High prevalence of fatty acid synthase expression in colorectal cancers in Middle Eastern patients and its potential role as a therapeutic target. *Am J Gastroenterol.* 2009; 104(7):1790–801. [PubMed: 19491830]

61. Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen JV, Smans K. Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. *Prog Lipid Res.* 2013; 52(4):585–9. [PubMed: 24001676]
62. Ogino S, Nishihara R, VanderWeele TJ, Wang M, Nishi A, Lochhead P, Qian ZR, Zhang X, Wu K, Nan H, Yoshida K, Milner DA Jr, Chan AT, Field AE, Camargo CA Jr, Williams MA, Giovannucci EL. Review Article: The Role of Molecular Pathological Epidemiology in the Study of Neoplastic and Non-neoplastic Diseases in the Era of Precision Medicine. *Epidemiology.* 2016; 27(4):602–11. [PubMed: 26928707]
63. Ikram MA, van der Lugt A, Niessen WJ, Koudstaal PJ, Krestin GP, Hofman A, Bos D, Vernooij MW. The Rotterdam Scan Study: design update 2016 and main findings. *Eur J Epidemiol.* 2015; 30(12):1299–315. [PubMed: 26650042]
64. Nishihara R, VanderWeele TJ, Shibuya K, Mittleman MA, Wang M, Field AE, Giovannucci E, Lochhead P, Ogino S. Molecular pathological epidemiology gives clues to paradoxical findings. *Eur J Epidemiol.* 2015; 30(10):1129–35. [PubMed: 26445996]
65. Snijder MB, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? *Int J Epidemiol.* 2006; 35(1):83–92. [PubMed: 16339600]

Abbreviations

AHEI	Alternate Healthy Eating Index
BMI	body mass index
CI	confidence interval
FFPE	formalin-fixed paraffin-embedded
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
ISC	intestinal stem cells
METS	metabolic equivalent task score
MPE	Molecular Pathological Epidemiology
MSI	microsatellite instability
MSS	microsatellite stable
NHS	Nurses' Health Study
NSAIDs	non-steroidal anti-inflammatory drugs
SD	standard deviation
WHO	World Health Organization

Table 1
Age-standardized characteristics of the Nurses' Health Study (NHS) (1980-2010) and the Health Professionals Follow-up Study (HPFS) (1986-2010) participants according to body mass index (person-years)*

Characteristics	Women (NHS)						Men (HPFS)					
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30	18.5-22.4	22.5-24.9
Participants, person-years	352576	323576	228396	135304	173152	59433	161809	154675	73416	45684		
Age, year [†]	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)	63.5 (10.9)	63.4 (10.3)	63.0 (9.9)		
Family history of colorectal 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 nonsteroidal anti-inflammatory drugs(NSAIDs), %	14.3	17.0	18.4	19.9	21.5	11.3	13.7	15.3	17.5	19.3		
Regular use of aspirin, %	32.2	33.5	35.3	36.2	37.8	41.2	45.6	47.6	49.1	49.7		
Pack-years 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)		
Ever smokers, %	58.1	56.7	55.4	53.9	51.9	50.1	54.8	59.1	60.1	61.6		
Total calorie, kcal/day	1665 (442)	1662 (435)	1667 (441)	1682 (447)	1716 (460)	2002 (544)	1967 (544)	1964 (561)	1977 (571)	2001 (586)		
Alcohol intake, g/day	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, metabolic equivalent task score (METs)-hours/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 (29.4)	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/day	927 (367)	926 (353)	919 (347)	914 (344)	896 (340)	948 (389)	935 (378)	918 (368)	920 (366)	927 (374)		
Folate, µg/day	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 [‡]	46.4 (10.1)	46.4 (9.7)	46.3 (9.4)	46.1 (9.2)	45.2 (9.3)	49.7 (11.0)	48.9 (10.2)	47.6 (9.8)	46.9 (9.5)	46.2 (9.6)		

* Updated information throughout follow-up was used to calculate the mean (standard deviation [SD]) for continuous variables and percentage for categorical variables.

[†] All values other than age have been directly standardized to age distribution (in 5-year age group) of all the participants.

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[‡]Without alcohol intake.

AHEI, Alternate Healthy Eating Index; HPFS, Health Professionals Follow-up Study; METS, metabolic equivalent task score; NHS, Nurses' Health Study; NSAIDs, nonsteroidal anti-inflammatory drugs.

Body mass index and risk of colorectal cancer overall and by tumor histopathological characteristics in the Nurses' Health Study (NHS)

Table 2

	Body mass index (kg/m ²)					P _{trend}	P _{heterogeneity} [‡]
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30		
Total colorectal cancer							
Person-years	699109	639958	450110	266076	339690		
Cases, No. (n=860)	177	222	187	111	163		
Age-adjusted HR (95% CI)	1 [Reference]	1.19 (0.98, 1.45)	1.34 (1.09, 1.64)	1.29 (1.02, 1.64)	1.58 (1.28, 1.96)	<0.001	
Multivariable HR (95% CI) [*]	1 [Reference]	1.18 (0.97, 1.45)	1.32 (1.07, 1.62)	1.27 (1.00, 1.62)	1.55 (1.24, 1.94)	<0.001	
Poorly-differentiated foci							
Absent							
Cases, No. (n=592)	114	146	132	78	122		
Age-adjusted HR (95% CI)	1 [Reference]	1.24 (0.97, 1.59)	1.50 (1.16, 1.93)	1.45 (1.09, 1.94)	1.87 (1.44, 2.41)	<0.001	0.02
Multivariable HR (95% CI) [*]	1 [Reference]	1.23 (0.96, 1.58)	1.47 (1.14, 1.90)	1.42 (1.06, 1.91)	1.82 (1.40, 2.38)	<0.001	0.02
Present							
Cases, No. (n=259)	60	74	52	33	40		
Age-adjusted HR (95% CI)	1 [Reference]	1.13 (0.80, 1.59)	1.04 (0.72, 1.51)	1.06 (0.69, 1.63)	1.10 (0.74, 1.65)	0.77	
Multivariable HR (95% CI) [*]	1 [Reference]	1.12 (0.79, 1.57)	1.03 (0.71, 1.49)	1.04 (0.67, 1.59)	1.08 (0.72, 1.63)	0.86	
Overall tumor differentiation							
Well/moderate							
Cases, No. (n=750)	150	187	169	98	146		
Age-adjusted HR (95% CI)	1 [Reference]	1.19 (0.96, 1.48)	1.43 (1.15, 1.78)	1.35 (1.05, 1.75)	1.67 (1.33, 2.11)	<0.001	0.08
Multivariable HR (95% CI) [*]	1 [Reference]	1.18 (0.95, 1.47)	1.41 (1.12, 1.76)	1.33 (1.03, 1.73)	1.64 (1.29, 2.09)	<0.001	0.08
Poor							
Cases, No. (n=104)	26	33	16	13	16		
Age-adjusted HR (95% CI)	1 [Reference]	1.16 (0.69, 1.94)	0.75 (0.40, 1.41)	0.98 (0.50, 1.91)	1.03 (0.55, 1.93)	0.82	
Multivariable HR (95% CI) [*]	1 [Reference]	1.15 (0.69, 1.93)	0.75 (0.40, 1.41)	0.95 (0.48, 1.86)	1.02 (0.54, 1.91)	0.77	
Mucinous component							

	Body mass index (kg/m ²)				P _{trend}	P _{heterogeneity} [†]
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30	
Absent (0%)						
Cases, No. (n=491)	108	119	108	60	96	
Age-adjusted HR (95% CI)	1 [Reference]	1.08 (0.83, 1.40)	1.30 (0.99, 1.70)	1.21 (0.88, 1.66)	1.59 (1.21, 2.10)	0.74
Multivariable HR (95% CI) [*]	1 [Reference]	1.07 (0.82, 1.39)	1.28 (0.98, 1.68)	1.19 (0.86, 1.63)	1.56 (1.17, 2.07)	0.75
1% to 49%						
Cases, No. (n=225)	48	62	44	32	39	
Age-adjusted HR (95% CI)	1 [Reference]	1.20 (0.82, 1.75)	1.13 (0.75, 1.70)	1.29 (0.82, 2.02)	1.34 (0.87, 2.05)	0.19
Multivariable HR (95% CI) [*]	1 [Reference]	1.20 (0.82, 1.75)	1.12 (0.74, 1.69)	1.26 (0.80, 1.98)	1.32 (0.86, 2.03)	0.24
50%						
Cases, No. (n=104)	17	26	23	14	24	
Age-adjusted HR (95% CI)	1 [Reference]	1.36 (0.74, 2.52)	1.58 (0.84, 2.96)	1.51 (0.74, 3.08)	2.21 (1.18, 4.12)	0.01
Multivariable HR (95% CI) [*]	1 [Reference]	1.35 (0.73, 2.49)	1.54 (0.82, 2.89)	1.48 (0.73, 3.02)	2.15 (1.15, 4.03)	0.02
Signet ring cell component						
Absent						
Cases, No. (n=750)	155	191	164	96	144	
Age-adjusted HR (95% CI)	1 [Reference]	1.19 (0.96, 1.47)	1.36 (1.09, 1.69)	1.30 (1.01, 1.68)	1.63 (1.29, 2.04)	0.39
Multivariable HR (95% CI) [*]	1 [Reference]	1.18 (0.95, 1.46)	1.34 (1.07, 1.67)	1.28 (0.99, 1.66)	1.60 (1.26, 2.03)	0.38
Present						
Cases, No. (n=110)	22	31	23	15	19	
Age-adjusted HR (95% CI)	1 [Reference]	1.23 (0.71, 2.13)	1.19 (0.66, 2.15)	1.21 (0.62, 2.34)	1.28 (0.69, 2.38)	0.51
Multivariable HR (95% CI) [*]	1 [Reference]	1.21 (0.70, 2.10)	1.18 (0.66, 2.13)	1.17 (0.61, 2.27)	1.26 (0.68, 2.34)	0.55

^{*} Adjusted for family history of colorectal cancer (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]-hours per week), red and processed meat intake (quintiles of servings per day), alcohol consumption (0, <5, 5-15, 15-30, 30+ gram per day), current multivitamin use (yes or no), regular use of aspirin (yes or no), regular use of non-steroidal 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 women, we additionally adjusted for menopause/postmenopausal hormone use status (premenopausal, postmenopausal never use, postmenopausal current hormone use).

[†] 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}.

CI, confidence interval; HR, hazard ratio; NHS, Nurses' Health Study.

Body mass index and risk of colorectal cancer overall and by tumor histopathological characteristics in the Health Professionals Follow-up Study (HPFS)

Table 3

	Body mass index (kg/m ²)				P _{trend}	P _{heterogeneity} [†]
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9		
Total colorectal cancer						
Person-years	59433	161809	154675	73416	45684	
Cases, No. (n=668)	58	202	221	117	70	
Age-adjusted HR (95% CI)	1 [Reference]	1.34 (1.00, 1.80)	1.55 (1.16, 2.08)	1.77 (1.28, 2.43)	1.76 (1.24, 2.51)	<0.001
Multivariable HR (95% CI) [*]	1 [Reference]	1.33 (0.99, 1.79)	1.52 (1.13, 2.04)	1.70 (1.23, 2.35)	1.71 (1.19, 2.46)	<0.001
Poorly-differentiated foci						
Absent						
Cases, No. (n=477)	40	140	156	89	52	
Age-adjusted HR (95% CI)	1 [Reference]	1.37 (0.96, 1.95)	1.62 (1.14, 2.30)	1.98 (1.36, 2.89)	1.95 (1.28, 2.97)	<0.001
Multivariable HR (95% CI) [*]	1 [Reference]	1.36 (0.95, 1.93)	1.58 (1.11, 2.25)	1.90 (1.29, 2.78)	1.89 (1.24, 2.90)	0.19
Present						
Cases, No. (n=178)	18	58	59	25	18	
Age-adjusted HR (95% CI)	1 [Reference]	1.19 (0.70, 2.04)	1.30 (0.76, 2.22)	1.17 (0.63, 2.16)	1.39 (0.71, 2.69)	0.42
Multivariable HR (95% CI) [*]	1 [Reference]	1.20 (0.70, 2.05)	1.26 (0.74, 2.16)	1.14 (0.61, 2.10)	1.36 (0.70, 2.66)	0.51
Overall tumor differentiation						
Well/moderate						
Cases, No. (n=616)	55	181	204	109	67	
Age-adjusted HR (95% CI)	1 [Reference]	1.27 (0.94, 1.72)	1.52 (1.13, 2.06)	1.76 (1.26, 2.44)	1.79 (1.25, 2.57)	<0.001
Multivariable HR (95% CI) [*]	1 [Reference]	1.26 (0.93, 1.71)	1.48 (1.09, 2.00)	1.68 (1.20, 2.34)	1.73 (1.19, 2.51)	0.31
Poor						
Cases, No. (n=45)	3	18	14	7	3	
Age-adjusted HR (95% CI)	1 [Reference]	2.21 (0.65, 7.55)	1.77 (0.50, 6.20)	1.77 (0.45, 6.91)	1.41 (0.28, 7.08)	0.90
Multivariable HR (95% CI) [*]	1 [Reference]	2.30 (0.67, 7.90)	1.83 (0.52, 6.47)	1.85 (0.47, 7.28)	1.45 (0.29, 7.31)	0.93

		Body mass index (kg/m ²)				P _{trend}	P _{heterogeneity} [†]
		18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30	
Mucinous component							
Absent (0%)							
Cases, No. (n=384)		34	125	125	64	36	
Age-adjusted HR (95% CI)		1 [Reference]	1.44 (0.98, 2.11)	1.48 (1.01, 2.18)	1.69 (1.11, 2.58)	1.57 (0.97, 2.52)	0.06
Multivariable HR (95% CI) [*]		1 [Reference]	1.43 (0.97, 2.10)	1.44 (0.98, 2.12)	1.60 (1.04, 2.45)	1.52 (0.94, 2.46)	0.11
1% to 49%							
Cases, No. (n=175)		15	51	60	33	16	
Age-adjusted HR (95% CI)		1 [Reference]	1.28 (0.72, 2.29)	1.70 (0.96, 3.01)	1.98 (1.07, 3.67)	1.51 (0.73, 3.10)	0.06
Multivariable HR (95% CI) [*]		1 [Reference]	1.25 (0.70, 2.23)	1.61 (0.91, 2.86)	1.87 (1.01, 3.48)	1.42 (0.69, 2.95)	0.09
50%							
Cases, No. (n=60)		4	18	20	6	12	
Age-adjusted HR (95% CI)		1 [Reference]	1.84 (0.62, 5.48)	2.20 (0.74, 6.50)	1.27 (0.35, 4.53)	4.63 (1.46, 14.7)	0.02
Multivariable HR (95% CI) [*]		1 [Reference]	1.91 (0.64, 5.68)	2.21 (0.75, 6.55)	1.22 (0.34, 4.38)	4.67 (1.46, 14.9)	0.03
Signet ring cell component							
Absent							
Cases, No. (n=603)		53	183	200	108	59	
Age-adjusted HR (95% CI)		1 [Reference]	1.32 (0.97, 1.80)	1.54 (1.13, 2.09)	1.79 (1.28, 2.49)	1.64 (1.12, 2.39)	<0.001
Multivariable HR (95% CI) [*]		1 [Reference]	1.32 (0.97, 1.80)	1.50 (1.10, 2.05)	1.72 (1.23, 2.41)	1.59 (1.08, 2.34)	0.003
Present							
Cases, No. (n=65)		5	19	21	9	11	
Age-adjusted HR (95% CI)		1 [Reference]	1.51 (0.56, 4.05)	1.73 (0.65, 4.63)	1.56 (0.52, 4.71)	2.95 (1.01, 8.61)	0.05
Multivariable HR (95% CI) [*]		1 [Reference]	1.46 (0.54, 3.94)	1.65 (0.62, 4.42)	1.49 (0.49, 4.50)	2.86 (0.98, 8.40)	0.06

^{*} Adjusted for the same set of covariates as in Table 2.

[†] 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}.

CI, confidence interval; HPFS, Health Professionals Follow-up Study; HR, hazard ratio.

Table 4
Body mass index and risk of colorectal cancer overall and by tumor histopathological characteristics in the pooled cohorts

	Body mass index (kg/m ²)					P _{trend}	P _{heterogeneity} [‡]
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30		
Total colorectal cancer							
Person-years	812884	951694	747717	407217	427160		
Cases, No. (n=1528)	235	424	408	228	233		
Age-adjusted HR (95% CI)	1 [Reference]	1.22 (1.04, 1.44)	1.39 (1.18, 1.64)	1.46 (1.21, 1.75)	1.62 (1.35, 1.95)	<0.001	
Multivariable HR (95% CI) [*]	1 [Reference]	1.22 (1.04, 1.43)	1.38 (1.17, 1.63)	1.43 (1.19, 1.73)	1.61 (1.33, 1.95)	<0.001	
Poorly-differentiated foci							
<i>Absent</i>							
Cases, No. (n=1069)	154	286	288	167	174		
Age-adjusted HR (95% CI)	1 [Reference]	1.27 (1.04, 1.55)	1.52 (1.24, 1.85)	1.65 (1.32, 2.07)	1.88 (1.51, 2.35)	<0.001	0.006
Multivariable HR (95% CI) [*]	1 [Reference]	1.27 (1.04, 1.55)	1.50 (1.23, 1.84)	1.63 (1.30, 2.04)	1.87 (1.49, 2.34)	<0.001	0.006
<i>Present</i>							
Cases, No. (n=437)	78	132	111	58	58		
Age-adjusted HR (95% CI)	1 [Reference]	1.13 (0.85, 1.50)	1.13 (0.84, 1.52)	1.08 (0.77, 1.53)	1.17 (0.83, 1.65)	0.51	
Multivariable HR (95% CI) [*]	1 [Reference]	1.13 (0.85, 1.50)	1.11 (0.83, 1.49)	1.06 (0.75, 1.50)	1.17 (0.82, 1.65)	0.56	
Overall tumor differentiation							
<i>Well/moderate</i>							
Cases, No. (n=1366)	205	368	373	207	213		
Age-adjusted HR (95% CI)	1 [Reference]	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	0.03
Multivariable HR (95% CI) [*]	1 [Reference]	1.20 (1.01, 1.43)	1.43 (1.20, 1.70)	1.48 (1.22, 1.81)	1.69 (1.38, 2.06)	<0.001	0.04
<i>Poor</i>							
Cases, No. (n=149)	29	51	30	20	19		
Age-adjusted HR (95% CI)	1 [Reference]	1.28 (0.81, 2.03)	0.91 (0.54, 1.54)	1.06 (0.59, 1.90)	1.06 (0.59, 1.90)	0.79	
Multivariable HR (95% CI) [*]	1 [Reference]	1.28 (0.80, 2.03)	0.92 (0.55, 1.55)	1.05 (0.59, 1.87)	1.07 (0.59, 1.91)	0.80	
Mucinous component							

	Body mass index (kg/m ²)					P _{trend}	P _{heterogeneity} [†]
	18.5-22.4	22.5-24.9	25.0-27.4	27.5-29.9	30		
Absent (0%)							
Cases, No. (n=875)	142	244	233	124	132		
Age-adjusted HR (95% CI)	1 [Reference]	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) *	1 [Reference]	1.19 (0.97, 1.47)	1.32 (1.06, 1.64)	1.34 (1.05, 1.72)	1.56 (1.23, 2.00)	<0.001	0.36
1% to 49%							
Cases, No. (n=400)	63	113	104	65	55		
Age-adjusted HR (95% CI)	1 [Reference]	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) *	1 [Reference]	1.18 (0.86, 1.61)	1.31 (0.95, 1.80)	1.48 (1.04, 2.11)	1.36 (0.94, 1.97)	0.04	
50%							
Cases, No. (n=164)	21	44	43	20	36		
Age-adjusted HR (95% CI)	1 [Reference]	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) *	1 [Reference]	1.46 (0.86, 2.46)	1.68 (0.99, 2.86)	1.34 (0.72, 2.49)	2.63 (1.53, 4.54)	0.001	
Signet ring cell component							
Absent							
Cases, No. (n=1353)	208	374	364	204	203		
Age-adjusted HR (95% CI)	1 [Reference]	1.22 (1.03, 1.45)	1.40 (1.18, 1.67)	1.48 (1.22, 1.80)	1.62 (1.34, 1.97)	<0.001	0.67
Multivariable HR (95% CI) *	1 [Reference]	1.22 (1.02, 1.45)	1.39 (1.17, 1.66)	1.46 (1.20, 1.78)	1.61 (1.32, 1.97)	<0.001	0.67
Present							
Cases, No. (n=175)	27	50	44	24	30		
Age-adjusted HR (95% CI)	1 [Reference]	1.25 (0.78, 2.01)	1.30 (0.80, 2.12)	1.26 (0.72, 2.19)	1.60 (0.95, 2.71)	0.13	
Multivariable HR (95% CI) *	1 [Reference]	1.23 (0.77, 1.98)	1.28 (0.79, 2.09)	1.23 (0.70, 2.14)	1.58 (0.93, 2.67)	0.14	

* Adjusted for the same set of covariates as in Table 2.

[†] 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}.

CI, confidence interval; HR, hazard ratio.