Prospective Analysis of Body Mass Index, Physical Activity, and Colorectal Cancer Risk Associated with β-Catenin (CTNNB1) Status

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Abstract

Dysregulation of the WNT/β-catenin (CTNNB1) signaling pathway is implicated in colorectal carcinoma and metabolic diseases. Considering these roles and cancer prevention, we hypothesized that tumor CTNNB1 status might influence cellular sensitivity to obesity and physical activity. In clinical follow-up of 109,046 women in the Nurses' Health Study and 47,684 men in the Health Professionals Follow-up Study, there were 861 incident rectal and colon cancers with tissue immunohistochemistry data on nuclear CTNNB1 expression. Using this molecular pathological epidemiology database, we conducted Cox proportional hazards regression analysis using data duplication method to assess differential associations of body mass index (BMI) or exercise activity with colorectal cancer risk according to tumor CTNNB1 status. Greater BMI was associated with a significantly higher risk of CTNNB1-negative cancer [multivariate HR = 1.34; 95% confidence interval (CI), 1.18–1.53 for 5.0 kg/m^2 increment; P_{trend} = 0.0001] but not with CTNNB1-positive cancer risk (multivariate HR = 1.07; 95% CI, 0.92–1.25 for 5.0 kg/m^2 increment; P_{trend} = 0.36; $P_{heterogeneity}$ = 0.027, between CTNNB1-negative and CTNNB1-positive cancer risks). Physical activity level was associated with a lower risk of CTNNB1-negative cancer (multivariate HR = 0.93; 95% CI, 0.87–1.00 for 10 MET-h/wk increment; P_{trend} = 0.044) but not with CTNNB1-positive cancer risk (multivariate HR = 0.98; 95% CI, 0.91–1.05 for 10 MET-h/wk increment; P_{trend} = 0.60). Our findings argue that obesity and physical inactivity are associated with a higher risk of CTNNB1-negative colorectal cancer but not with CTNNB1-positive cancer risk. Furthermore, they suggest that energy balance and metabolism status exerts its effect in a specific carcinogenesis pathway that is less likely dependent on WNT/CTNNB1 activation. *Cancer Res; 73(5); 1600–10. ©2012 AACR.*

Introduction

Accumulating evidence suggests that excess adiposity may be causally linked to colorectal cancer (1–4), and that physical activity may decrease colorectal cancer risk (5–8). Although energy metabolism likely plays an important role in colorectal carcinogenesis (9, 10), the underlying mechanisms linking obesity or physical inactivity and cancer remain to be fully elucidated. A better understanding of the effect of obesity and physical activity on carcinogenic pathways may help us develop better cancer prevention strategies.

Activation of the WNT signaling pathway (most commonly resulting from *APC* loss) plays a critical role in colorectal carcinogenesis (11, 12). β-Catenin (CTNNB1; official HGNC ID: HGNC:2514) is a major mediator of the WNT pathway. Nuclear CTNNB1 binds the transcription factors lymphoid enhancer-binding factor 1 (LEF1) and T-cell factor (TCF), thereby converting LEF1 into a transcriptional activator (12). Overexpression of mitogenic WNT-CTNNB1–regulated genes such as *MYC* and *CCND1* (cyclin D1) contributes to tumor progression (12).

Accumulating evidence supports a role for WNT-CTNNB1 signaling in adipogenesis, obesity, glucose metabolism, and metabolic disease (12–14). Metabolic diseases such as obesity and type II diabetes are influenced by genetic and functional variations in the WNT signaling pathway. In addition, evidence suggests that obesity and physical activity modulate WNT-CTNNB1 signaling in the mouse colonic mucosa (15, 16). Considering the multifaceted roles of

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CTNNB1 in carcinogenesis and energy metabolism, we hypothesized that the association of obesity and physical activity with colorectal cancer risk might differ by tumor subtypes according to CTNNB1 status. To test this hypothesis, we used 2 U.S. nationwide prospective cohort studies with available CTNNB1 expression data in incident colorectal cancers. Our findings suggest a role for energy metabolism in a specific molecular carcinogenic pathway that is less dependent on WNT/CTNNB1 activation.

Materials and Methods

Study population

We used a database of 2 prospective cohort studies, the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS; refs. 17, 18). We used data from questionnaires, which were sent to participants every 2 years to update information on weight, physical activity, diet, smoking status, and other lifestyle factors, and to identify newly diagnosed cancers in participants and their first-degree relatives. Follow-up rates of the cohorts were more than 90%. In the present study, exclusion criteria were as follows: a personal history of inflammatory bowel disease or cancer (except non-melanoma skin cancer) before 1986 and incomplete data on weight or height. A total of 47,684 men and 109,046 women were eligible for the analysis.

Informed consent was obtained from all participants in this study. This study was approved by the Human Subjects Committees at Harvard School of Public Health (Boston, MA) and Brigham and Women's Hospital (Boston, MA).

Weight and height information

Weight information (weight measured in pounds at a given time point) was first collected in 1986 for men and in 1976 for women, and updated every 2 years. A validation study has shown that, in these cohorts, self-reported weight is highly accurate as compared with standardized measurements (19). In the validation study (19), trained technicians visited the substudy participants, approximately every 6 months, to measure their weight. The Pearson correlation coefficient between the self-reported weight and the mean of the technicians' 2 measurements was 0.97 (19).

To assess the influence of body mass index (BMI) on subsequent cancer incidence, we used height reported in 1986 for men and in 1976 for women, 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 (20). We evaluated the association between the cumulative mean BMI and the risk of colorectal cancer in the next 2-year follow-up period.

Assessment of physical activity

Leisure-time physical activity was assessed every 2 years in both cohorts, as previously described, and validated against subject diaries (21). Subjects reported the amount of time (ranging from 0 to 11 or more h/wk) spent engaged in walking (at usual pace), jogging, running, bicycling, swimming laps, racket sports, other aerobic exercises, low intensity exercise (yoga, toning, stretching, etc.), and other vigorous activities. Each activity on the questionnaire was assigned a metabolic equivalent task (MET) score (22). One MET is equivalent to energy expenditure at rest. MET scores for specific activities represent the activity-related metabolic rate divided by the resting metabolic rate. In the present study, values for individual activities were summed to give a total MET-h/wk score.

To assess the influence of physical activity on subsequent cancer incidence, we used the cumulative mean MET-h/wk score, which was the mean of all available physical activity data up to the start of each 2-year follow-up period.

Ascertainment and pathologic examination of colorectal cancers

We requested consent to review medical records and pathology reports for participants who reported colorectal cancer on their biennial questionnaire (18). The National Death Index was used to identify unreported cases of lethal cancer. Study investigators reviewed these medical records and extracted information on pathologic stage and anatomic location of the cancers (23). We included both colon and rectal cancers because of continuum of features of colorectal cancers from rectum to ascending colon (23, 24). We collected available paraffin-embedded tissue blocks from hospitals throughout the United States, where participants had undergone tumor resection (17). Tissue sections from colorectal cancer cases (i.e., all incident colorectal cancer events in the current study) were reviewed and confirmed by a pathologist (S. Ogino; ref. 23).

Immunohistochemistry for CTNNB1

Tissue microarrays were constructed (25), and immunohistochemistry for CTNNB1 (β-catenin; HGNC ID, 2514) expression in colorectal cancer was conducted, as previously described (17, 26). A pathologist (T. Morikawa), blinded to other study data, interpreted CTNNB1 expression in all cases. CTNNB1 expression status was categorized as negative (if there was weak or no nuclear expression), or positive (if there was moderate or strong nuclear expression). Our method for validation of the cutoff has been described previously (17). For the agreement study, a random selection of 292 cancers was evaluated by a second pathologist (S. Ogino), blinded to all other data. The concordance between the 2 observers was 0.90 ($\kappa = 0.80$; $P < 0.0001$), indicating substantial agreement.

During follow-up, 2,263 incident colorectal cancers (842 in men and 1,421 in women) were identified between 1986 and 2004. On the basis of tissue sample availability, we used CTNNB1 expression data from 861 incident colorectal cancers (368 in men and 493 in women). The baseline characteristics of participants with CTNNB1 expression data were generally similar to those of participants lacking CTNNB1 expression data: BMI (kg/m^2), 25.4 versus 25.4; mean age (years), 57.4 versus 57.2; past or current smoking, 60% versus 58%; physical activity (MET-h/wk), 17.0 versus 15.0; postmenopausal women (in NHS), 78% versus 79%; family history of colorectal cancer in any first-degree relative, 13% versus 11%; history of sigmoidoscopy or colonoscopy, 18% versus 17%; current multivitamin use, 37% versus 35%; regular aspirin use, 29% versus 26%; mean

caloric intake (kcal/d), 1,821 versus 1,812; mean alcohol intake (g/d), 10.4 versus 9.6; mean folate intake (µg/d), 407 versus 415; mean vitamin D intake (IU/d), 346 versus 360; mean calcium intake (mg/d), 863 versus 869; mean red meat intake (servings/d), 1.2 versus 1.2; $P > 0.05$ for all comparisons.

Statistical analysis

Follow-up time for an incident event was calculated as the time from the date of return of the 1986 questionnaire to the date of first colorectal cancer diagnosis, date of censoring due to death from other causes, or June 2004, whichever came first. For colorectal cancer cases where we could not assess CTNNB1 expression, observations were censored at the time of diagnosis.

For potential confounding variables, age-standardized mean values and proportions were computed for each BMI category at baseline in the both cohorts. For dietary intake in the NHS, cumulative mean values from 1980 to 1986 were used as the baseline data. Cox proportional hazards model was used to estimate the HR and 95% confidence interval (CI), adjusted for potential confounders including physical activity (MET-h/wk in quintiles), energy-adjusted folate, vitamin D and calcium intakes (in quintiles), total caloric intake (continuous), red meat intake (in quintiles), current smoking status (current, past, and never), pack-year of smoking before 30 years of age (0, 1–4, 5–10, 11, or more), alcohol intake (0, 0.1–4.9, 5–14.9, $\geq$15 g/d), current multivitamin use (yes or no), regular aspirin use (yes or no), previous sigmoidoscopy or colonoscopy (never or ever), family history of colorectal cancer in any first-degree relative (yes or no), and menopausal status/postmenopausal hormone therapy (HT) use (for women only; premenopausal, postmenopausal/HT never use, postmenopausal/HT past use, postmenopausal/HT current use). We chose these covariates based on our previous data, which have shown these factors to be associated with colorectal cancer risk (27). The analysis was stratified by 1-year age because age is a strong predictor of colorectal cancer incidence. Proportionality of hazards assumptions was assessed by a time-varying covariate (an interaction term of survival time and BMI, $P = 0.072$ for CTNNB1-positive cancer event, $P = 0.47$ for CTNNB1-negative cancer event; an interaction term of survival time and physical activity, $P = 0.72$ for CTNNB1-positive cancer event, $P = 0.043$ for CTNNB1-negative cancer event).

To compare the influences of BMI and physical activity on CTNNB1-positive colorectal cancer risk versus CTNNB1-negative colorectal cancer risk, we used a data duplication method in Cox proportional hazards model (25). This method provides separate regression coefficients by CTNNB1 status. We assessed the difference between the HR estimates according to tumor CTNNB1 status by a likelihood ratio test. This compared a model that allowed for separate associations (of BMI and physical activity with cancer risk) by CTNNB1 status, with a model that assumed a common association. To obtain a P_{trend} or $P_{heterogeneity}$, we used the median BMI value of each BMI category or median MET-h/wk score of each physical activity category as one numeric variable. To obtain a P_{trend} or $P_{heterogeneity}$ in the combined analysis of BMI and physical activity, each of the 4 categories (i.e., low BMI/high physical

activity, low BMI/low physical activity, high BMI/high physical activity, or high BMI/low physical activity) was coded as 0, 1, 1, or 2, respectively. We conducted the Q test to assess heterogeneity in the trend effects of BMI and physical activity on the risk of each cancer subtype (CTNNB1-positive or -negative) between the 2 cohorts. We used SAS software (version 9.2, SAS Institute) for all analyses. All P values were two-sided.

Results

Characteristics of colorectal cancer patient cohorts

Tables 1 and 2 show the age-standardized characteristics of the study population according to baseline BMI in men (HPFS) and women (NHS), respectively. BMI was inversely associated with physical activity level and current multivitamin use and positively associated with regular aspirin use in both cohorts. We categorized BMI according to the World Health Organization (WHO) classification system (<18.5, 18.5–22.9, 23.0–24.9, 25.0–27.4, 27.5–29.9, $\geq$30 kg/m^2).

After follow-up (a total of 2,631,423 person-years) of 47,684 men and 109,046 women, there were 861 incident colorectal cancers with available tumor CTNNB1 expression data; 467 tumors (54%) were nuclear CTNNB1-negative and 394 tumors (46%) were nuclear CTNNB1-positive. Nuclear CTNNB1-positivity was observed in 183 of 368 tumors (50%) in men and 209 of 493 tumors (42%) in women. Nuclear CTNNB1-positivity was found in 129 of 399 tumors (32%) in the proximal colon, 148 of 266 tumors (56%) in the distal colon, and 177 of 196 tumors (60%) in the rectum. Nuclear CTNNB1 positivity was not observed in adjacent normal mucosal specimens. The associations between the baseline characteristics and tumor CTNNB1 status are shown in Supplementary Table S1.

BMI and the risk of incident colorectal cancer according to tumor CTNNB1 status

Table 3 shows the association between the cumulative mean BMI and the risk of developing colorectal cancer according to CTNNB1 status in men and women. In men, higher BMI was associated with a significantly higher risk of CTNNB1-negative colorectal cancer (multivariate HR = 1.41; 95% CI, 1.08–1.82 for a 5.0 kg/m^2 increment; $P_{trend} = 0.01$). Compared with men having BMI of 18.5–22.9 kg/m^2, multivariate HR for BMI of 27.5–29.9 kg/m^2, or BMI of $\geq$30 kg/m^2, was 1.88 (95% CI, 1.08–3.26) and 2.00 (95% CI, 1.07–3.73), respectively. In contrast, increasing BMI was not associated with CTNNB1-positive colorectal cancer risk (multivariate HR = 0.94; 95% CI, 0.71–1.24 for a 5.0 kg/m^2 increment; $P_{trend} = 0.64$; $P_{heterogeneity} = 0.033$, between CTNNB1-negative and CTNNB1-positive cancer risks).

Similarly, in women, higher BMI was associated with a significantly higher risk of CTNNB1-negative colorectal cancer (multivariate HR = 1.29; 95% CI, 1.11–1.50 for a 5.0 kg/m^2 increment; $P_{trend} = 0.0009$; Table 3). In contrast, increasing BMI was not associated with increased risk of CTNNB1-positive tumors (multivariate HR = 1.12; 95% CI, 0.93–1.34 for a 5.0 kg/m^2 increment; $P_{trend} = 0.23$; $P_{heterogeneity} = 0.22$, between CTNNB1-negative and CTNNB1-positive cancer risks).

There was no significant heterogeneity in the association of BMI with the risk of each cancer subtype (CTNNB1-positive or

Table 1. Age-standardized characteristics (1986) of the study population in men (the HPFS) according to BMI in 1986

Characteristics[a]	BMI (kg/m^2)					
	<18.5	18.5–22.9	23.0–24.9	25.0–27.4	27.5–29.9	≥30
Participants (no.)	165	8,934	13,302	15,416	6,044	3,823
Age	56.9 (11.0)	53.9 (10.3)	54.2 (9.9)	54.8 (9.6)	54.8 (9.3)	54.3 (9.0)
Past or current smoker (%)	56	46	50	53	54	56
Physical activity[b] (MET/wk)	17.6 (29.6)	25.6 (34.0)	23.4 (31.4)	20.1 (26.9)	16.5 (24.6)	13.1 (25.4)
Family history of colorectal cancer in any first-degree relative (%)	8	8	8	9	8	9
History of sigmoidoscopy (%)	21	24	24	22	22	23
Current multivitamin use (%)	43	47	44	41	36	36
Current aspirin use (%)	26	27	29	30	31	32
Dietary intakes						
Calories (kcal/d)	2,059 (637)	2,005 (606)	1,980 (606)	1,969 (620)	1,987 (638)	2,018 (656)
Alcohol (g/d)	11.8 (17.2)	10.7 (14.5)	11.6 (15.1)	11.6 (15.6)	11.6 (16.3)	10.2 (16.2)
Folate[c] (μg/d)	457 (245)	508 (290)	495 (285)	470 (268)	451 (254)	450 (270)
Vitamin D[c] (IU/d)	370 (272)	429 (322)	420 (322)	399 (305)	380 (295)	384 (302)
Calcium[c] (mg/d)	895 (483)	915 (429)	907 (430)	887 (421)	884 (421)	894 (425)
Red meat (servings/d)	1.1 (0.9)	1.0 (0.8)	1.1 (0.8)	1.2 (0.8)	1.3 (0.9)	1.4 (0.9)

[a]Mean (SD), unless other specified.
[b]MET-hours, sum of average time/wk spent in each activity multiplied by the MET value of each activity.
[c]Energy-adjusted intakes.

negative) between the 2 cohorts ($P > 0.29$ by Q test). For further analyses, we combined the 2 cohorts to increase statistical power.

Table 4 shows the association between the cumulative mean BMI and the risk of colorectal cancer according to CTNNB1 status in the combined cohort of men and women. Increasing BMI was associated with a significantly higher risk of CTNNB1-negative cancer (multivariate HR = 1.34; 95% CI, 1.18–1.53, for a 5.0 kg/m^2 increment; $P_{trend} < 0.0001$). Compared with patients with BMI of 18.5–22.9 kg/m^2, multivariate HR for BMI of 27.5–29.9 kg/m^2, or BMI of ≥30 kg/m^2, was 1.77 (95% CI, 1.30–2.42), and 1.84 (95% CI, 1.34–2.53), respectively. In contrast, increasing BMI was not associated with increased risk of CTNNB1-positive tumors (multivariate HR = 1.07; 95% CI, 0.92–1.25, for a 5.0 kg/m^2 increment; $P_{trend} = 0.36$; $P_{heterogeneity} = 0.027$, between CTNNB1-negative and CTNNB1-positive cancer risks).

We conducted secondary analyses to assess associations between BMI and risk of colorectal cancer according to nuclear CTNNB1 status by tumor location (i.e., colon, proximal colon, distal colon, and rectum; Supplementary Tables S2 and S3). Although statistical power was limited in subsite analyses for proximal colon, distal colon, and rectal cancers, the results were generally similar to our main analysis findings for all tumor locations.

As a sensitivity analysis to minimize possible reverse causation (i.e., the effect of occult cancer on prediagnosis BMI), we examined (in the combined cohort) whether the association between baseline BMI (in 1986) and the risk of colorectal cancer, reported between 1990 and 2004, differed by tumor CTNNB1 status. Higher baseline BMI was associated with a significantly higher risk of CTNNB1-negative colorectal cancer (multivariate HR = 1.29; 95% CI, 1.12–1.47, for 5.0 kg/m^2 increment; $P_{trend} = 0.0002$). In contrast, higher baseline BMI was not associated with CTNNB1-positive cancer risk (multivariate HR = 1.06; 95% CI, 0.91–1.24, for 5.0 kg/m^2 increment; $P_{trend} = 0.46$; $P_{heterogeneity} = 0.063$, between CTNNB1-negative and CTNNB1-positive cancer risks).

Physical activity and the risk of colorectal cancer according to tumor CTNNB1 status

A total of 2,054,497 person-years and 767 incident colorectal cancers (364 in men and 403 in women) were available for the analyses of physical activity and incident cancer risk according to CTNNB1 status. Physical activity level seemed to be associated with a lower risk of CTNNB1-negative colorectal cancer in both cohorts, although statistical significance was not reached in men (Supplementary Table S4). In contrast, physical activity level was not associated with CTNNB1-positive cancer risk in either cohort. There was no significant heterogeneity in the association of physical activity level with the risk of each cancer subtype (CTNNB1-positive or negative) between the 2 cohorts ($P > 0.55$ by Q test). For further analyses, we combined the 2 cohorts to increase statistical power.

Table 5 shows the association between physical activity level and the risk of colorectal cancer according to CTNNB1 status in the combined cohort of men and women. Physical activity level was associated with a significantly lower risk of CTNNB1-negative cancer (multivariate HR = 0.93; 95% CI, 0.87–1.00 for

Table 2. Age-standardized characteristics (1986) of the study population in women (the NHS) according to cumulative mean BMI from 1976 to 1986

Characteristics[a]	BMI (kg/m^2)					
	<18.5	18.5–22.9	23.0–24.9	25.0–27.4	27.5–29.9	≥30
Participants (no.)	1,774	46,969	22,179	17,262	9,488	11,374
Age	50.7 (7.4)	51.0 (7.2)	52.8 (7.2)	53.4 (7.1)	53.7 (7.0)	53.2 (6.9)
Past and current smoker (%)	55	54	52	51	49	47
Physical activity[b] (MET/wk)	15.2 (22.9)	16.3 (23.3)	13.9 (19.3)	12.7 (19.2)	11.8 (19.1)	9.5 (14.3)
Menopausal status and hormone use (%)						
Premenopausal	26	31	32	31	31	31
Postmenopausal and never use	20	22	24	26	28	30
Postmenopausal and current use	15	16	14	13	11	8
Postmenopausal and past use	11	11	12	12	13	11
Family history of colorectal cancer in any first-degree relative (%)	6	7	7	7	7	7
History of sigmoidoscopy (%)	13	14	14	14	13	13
Current multivitamin use (%)	35	38	36	35	34	32
Current aspirin use[c] (%)	24	25	27	28	29	31
Dietary intakes						
Calories[d] (kcal/d)	1,739 (512)	1,672 (457)	1,666 (453)	1,667 (460)	1,694 (470)	1,730 (486)
Alcohol[d] (g/d)	7.3 (11.5)	7.7 (10.7)	6.7 (10.1)	5.5 (9.3)	4.8 (9.1)	3.2 (7.5)
Folate[d,e] (µg/d)	374 (199)	388 (213)	381 (206)	378 (200)	374 (226)	367 (203)
Vitamin D[d,e] (IU/d)	320 (217)	331 (228)	327 (222)	327 (219)	326 (243)	324 (223)
Calcium[d,e] (mg/d)	829 (336)	880 (343)	877 (335)	873 (333)	864 (330)	853 (344)
Red meat (servings/d)	1.3 (0.7)	1.2 (0.6)	1.2 (0.6)	1.3 (0.6)	1.3 (0.6)	1.4 (0.7)

[a]Mean (SD) unless other specified.
[b]MET-hours, sum of average time/wk spent in each activity multiplied by the MET value of each activity.
[c]Regular aspirin use in 1984.
[d]On the basis of cumulative mean from 1980 to 1986.
[e]Energy-adjusted intakes.

10 MET-h/wk increment; $P_{trend} = 0.044$). Physical activity level was not associated with CTNNB1-positive cancer risk (multivariate HR = 0.98; 95% CI, 0.91–1.05 for 10 MET-h/wk increment; $P_{trend} = 0.60$); however, the association between physical activity and the cancer risk did not significantly differ by tumor CTNNB1 status ($P_{heterogeneity} = 0.29$).

BMI, physical activity, and the risk of colorectal cancer according to tumor CTNNB1 status

As an exploratory analysis, we examined an effect of BMI and physical activity on colorectal cancer risk according to tumor CTNNB1 status. We classified patients into 4 categories by physical activity [MET-h/wk, <9.0 (low) vs. ≥9.0 (high)], and BMI [<25.0 kg/m^2 (low) vs. ≥25.0 kg/m^2 (high); Table 6; Supplementary Table S5 for each cohort]. In the combined cohort of men and women, the risk of CTNNB1-negative colorectal cancer was highest in the high-BMI/low-activity category (multivariate HR = 1.82; 95% CI, 1.40–2.38; compared with the low-BMI/high-activity category; $P_{trend} < 0.0001$). In contrast, combined BMI/physical activity status was not associated with CTNNB1-positive cancer risk ($P_{trend} = 0.22$). The association between combined BMI and physical activity status and cancer risk seemed to differ by CTNNB1 status,

although the difference was of borderline statistical significance ($P_{heterogeneity} = 0.05$).

Discussion

In the present study, we showed that increasing BMI was associated with a higher risk of CTNNB1-negative colorectal cancer but not with the risk of CTNNB1-positive cancer. In addition, physical activity level was associated with a lower risk of CTNNB1-negative colorectal cancer. These results seemed to be consistent between the 2 cohort studies. Our data support roles of obesity and low physical activity in the development of colorectal cancer, which is less dependent on nuclear localization of CTNNB1.

There are several possible mechanisms through which obesity and low physical activity associate with increased risk of CTNNB1-negative colorectal cancer but not with that of CTNNB1-positive colorectal cancer. These mechanisms may not be mutually exclusive and some mechanisms may be operative together. First, obesity and low physical activity may cause certain genetic/epigenetic aberrations unrelated to the WNT-CTNNB1 pathway (leading to the development of CTNNB1-negative colorectal cancer), whereas other etiologic factors may trigger activation of the WNT-CTNNB1 pathway

Table 3. BMI and colorectal cancer risk according to nuclear CTNNB1 expression status in men and women

	Cumulative mean BMI							
	<18.5	18.5–22.9	23.0–24.9	25.0–27.4	27.5–29.9	≥30	P_{trend}[a]	$P_{heterogeneity}$[b]
Men (HPFS)								
All colorectal cancers (N = 368)								
Cases/person-years	0/1,740	54/133,142	99/215,324	121/23,9503	60/110,178	34/67,656		
Age-adjusted HR (95% CI)		1 (referent)	1.17 (0.83–1.63)	1.26 (0.91–1.75)	1.38 (0.95–2.00)	1.28 (0.83–1.99)	0.13	
Multivariate HR[c] (95% CI)		1 (referent)	1.18 (0.84–1.65)	1.26 (0.91–1.75)	1.36 (0.93–1.98)	1.30 (0.83–2.03)	0.14	
CTNNB1 (−) colorectal cancers (N = 183)								
Cases/person-years	0/1740	22/133,168	46/215,374	61/239,559	34/110,204	20/67,667		
Age-adjusted HR (95% CI)		1 (referent)	1.38 (0.82–2.31)	1.65 (1.01–2.70)	1.93 (1.12–3.32)	1.96 (1.06–3.62)	0.009	
Multivariate HR[c] (95% CI)		1 (referent)	1.38 (0.82–2.32)	1.65 (1.00–2.71)	1.88 (1.08–3.26)	2.00 (1.07–3.73)	0.01	0.033
CTNNB1 (+) colorectal cancers (N = 185)								
Cases/person-years	0/1,740	32/133,159	53/215,368	60/239,561	26/110,205	14/67,673		
Age-adjusted HR (95% CI)		1 (referent)	1.02 (0.66–1.59)	1.01 (0.65–1.55)	1.01 (0.60–1.70)	0.83 (0.43–1.60)	0.65	
Multivariate HR[c] (95% CI)		1 (referent)	1.04 (0.67–1.62)	1.01 (0.65–1.56)	1.00 (0.59–1.70)	0.84 (0.44–1.62)	0.64	
Women (NHS)								
All colorectal cancers (N = 493)								
Cases/person-years	5/23,116	133/68,6716	100/38,7097	107/330,693	64/190,232	84/230,096		
Age-adjusted HR (95% CI)	1.14 (0.46–2.78)	1 (referent)	1.17 (0.90–1.51)	1.41 (1.09–1.82)	1.42 (1.06–1.92)	1.59 (1.21–2.09)	0.0003	
Multivariate HR[d] (95% CI)	1.18 (0.48–2.88)	1 (referent)	1.13 (0.87–1.46)	1.36 (1.05–1.76)	1.38 (1.02–1.87)	1.55 (1.16–2.06)	0.001	
CTNNB1 (−) colorectal cancers (N = 284)								
Cases/person-years	2/23,120	72/686,767	56/387,134	60/330,735	43/190,247	51/230,126		
Age-adjusted HR (95% CI)	0.82 (0.20–3.34)	1 (referent)	1.20 (0.84–1.70)	1.45 (1.03–2.05)	1.76 (1.20–2.57)	1.76 (1.23–2.52)	0.0003	
Multivariate HR[d] (95% CI)	0.84 (0.20–3.42)	1 (referent)	1.16 (0.82–1.65)	1.41 (1.00–1.99)	1.70 (1.16–2.49)	1.72 (1.18–2.49)	0.0009	0.22
CTNNB1 (+) colorectal cancers (N = 209)								
Cases/person-years	3/23,116	61/686,779	44/387,146	47/330,748	21/190,275	33/230,137		
Age-adjusted HR (95% CI)	1.55 (0.48–4.95)	1 (referent)	1.13 (0.76–1.66)	1.35 (0.92–1.98)	1.03 (0.62–1.69)	1.38 (0.90–2.11)	0.17	
Multivariate HR[d] (95% CI)	1.62 (0.51–5.20)	1 (referent)	1.08 (0.73–1.60)	1.31 (0.89–1.92)	1.00 (0.60–1.65)	1.34 (0.87–2.07)	0.23	

[a]P_{trend} test in participants with cumulative mean BMI ≥ 18.5 kg/m².
[b]$P_{heterogeneity}$ (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks in participants with cumulative mean BMI ≥ 18.5 kg/m².
[c]Adjusted for cumulative mean physical activity, alcohol, folate, vitamin D, calcium, caloric and red meat intake, current smoking status, smoking before 30 years of age, current multivitamin use, current aspirin use, previous sigmoidoscopy, and family history of colorectal cancer.
[d]Adjusted for the above-mentioned covariates and postmenopausal hormone therapy.

Table 4. BMI and colorectal cancer risk according to nuclear CTNNB1 expression status in the combined cohort of men and women

	Cumulative mean BMI						P_{trend}[a]	$P_{heterogeneity}$[b]
	<18.5	18.5–22.9	23.0–24.9	25.0–27.4	27.5–29.9	≥30		
All colorectal cancers (N = 861)								
Cases/person-years	5/24,884	187/82,2340	199/60,6719	228/57,5202	124/30,2938	118/29,9340		
Multivariate HR[c] (95% CI)	0.96 (0.39–2.35)	1 (referent)	1.17 (0.95–1.43)	1.34 (1.09–1.63)	1.41 (1.11–1.78)	1.52 (1.19–1.93)	0.0001	
CTNNB1 (−) colorectal cancers (N = 467)								
Cases/person-years	2/24,888	94/822,417	102/606,806	121/575,299	77/302,979	71/299,381		
Multivariate HR[c] (95% CI)	0.73 (0.18–2.97)	1 (referent)	1.23 (0.93–1.64)	1.49 (1.13–1.97)	1.77 (1.30–2.42)	1.84 (1.34–2.53)	<0.0001	0.027
CTNNB1 (+) colorectal cancers (N = 394)								
Cases/person-years	3/24,884	93/822,420	97/606,811	107/575,315	47/303,008	47/299,398		
Multivariate HR[c] (95% CI)	1.25 (0.39–3.96)	1 (referent)	1.10 (0.82–1.47)	1.18 (0.88–1.57)	1.05 (0.73–1.50)	1.19 (0.83–1.71)	0.36	

[a]P_{trend} test in participants with cumulative mean BMI ≥ 18.5 kg/m².
[b]$P_{heterogeneity}$ (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks in participants with cumulative mean BMI ≥ 18.5 kg/m².
[c]Adjusted for cumulative mean physical activity, alcohol, folate, vitamin D, calcium, caloric and red meat intake, current smoking status, smoking before 30 years of age, current multivitamin use, current aspirin use, previous sigmoidoscopy, and family history of colorectal cancer.

(leading to the development of CTNNB1-positive colorectal cancer). It remains to be elucidated which molecular changes are triggered by excess energy balance.

Second, through stroma–tumor interaction, energy balance status may differentially influence early neoplastic cells according to cellular CTNNB1 status, and CTNNB1-negative neoplasm may be driven by excess energy balance status. Our previous data suggest that postdiagnosis progression of CTNNB1-negative colorectal cancer is dependent on the patient's energy balance status, whereas CTNNB1-positive cancer may progress regardless of the patient's energy balance status (17). Together with our previous data, our current findings suggest that tumor CTNNB1 status may influence cellular sensitivity to obesity and physical activity not only during the progression of an established cancer but also during earlier steps of tumor development up to clinical detection (Fig. 1).

Accumulating evidence suggests that insulin and the insulin-like growth factor (IGF) axis are putative mediators of the causal link between obesity and colorectal cancer (9, 28). Increased circulating levels of insulin and free IGF1 are associated with obesity, physical inactivity, and an increased cancer risk (9, 28). In a number of cancer cell lines, insulin and IGF1 promote cell survival and proliferation, at least in part through transient activation of the CTNNB1 signaling pathway (12, 29, 30). A recent study using a mouse model also showed that high-fat diet-induced obesity resulted in increases in the expression of Ctnnb1 and its downstream target, oncogene *Myc*, in the normal colonic mucosa (15). Although nuclear CTNNB1 positivity, determined by immunohistochemistry, was not observed in normal colonic mucosa in the present study, the long-term effect of relatively low-level CTNNB1 signaling in colonic epithelial cells induced by obesity, insulin, or IGF1 may play an important role in colorectal carcinogenesis. In contrast, the behavior of neoplastic cells with constitutively activated CTNNB1 signaling, detectable by immunohistochemistry, may be less dependent on obesity, insulin, or IGF1. In fact, IGF1R expression level is high in colorectal cancer cells lacking *APC* or *CTNNB1* mutation, and the IGF1 signaling pathway seems to be more important in these cells compared with colorectal cancer cells with constitutively activated CTNNB1 signaling resulting from *APC* or *CTNNB1* mutation (29). Further studies are warranted to elucidate the exact mechanisms through which obesity, physical activity, insulin, and IGF1 influence tumor cell progression via modulation of CTNNB1 signaling.

Another possible explanation for our findings is that the WNT-CTNNB1 pathway may tend to be downregulated with the presence of excess energy balance status during colorectal carcinogenesis process (shifting CTNNB1-positive tumors into CTNNB1-negative tumors). A study showed that voluntary exercise decreased nuclear Ctnnb1 expression in mouse intestinal tumor (16). In this model, we should then expect a compensatory decrease in the risk of CTNNB1-positive cancers among obese participants. However, in the present study, the risk of nuclear CTNNB1-positive cancer was not decreased in obese participants (Table 4). A recent study using a mouse model showed that obesity resulted in increase in the expression of Ctnnb1 in the normal colonic mucosa (15). Therefore,

Table 5. Physical activity and colorectal cancer risk according to nuclear CTNNB1 expression status in the combined cohort of men and women

	Physical activity (MET-h/wk)						
	<3.0	3.0–8.9	9.0–17.9	18.0–26.9	≥27.0	P_{trend}	$P_{heterogeneity}$[a]
All colorectal cancers[b] (N = 767)							
Cases/person-years	92/255,551	179/463,566	187/491,022	108/307,464	201/536,894		
Multivariate HR[c] (95% CI)	1 (referent)	0.94 (0.73–1.22)	0.88 (0.68–1.14)	0.78 (0.58–1.04)	0.79 (0.61–1.04)	0.074	
CTNNB1 (−) colorectal cancers (N = 415)							
Cases/person-years	58/255,580	99/463,629	101/491,100	55/307,519	102/536,978		
Multivariate HR[c] (95% CI)	1 (referent)	0.85 (0.61–1.18)	0.76 (0.55–1.06)	0.64 (0.44–0.94)	0.68 (0.48–0.96)	0.044	0.29
CTNNB1 (+) colorectal cancers (N = 352)							
Cases/person-years	34/255,602	80/463,657	86/491,109	53/307,508	99/536,985		
Multivariate HR[c] (95% CI)	1 (referent)	1.11 (0.74–1.66)	1.08 (0.72–1.63)	1.01 (0.65–1.57)	0.98 (0.65–1.49)	0.60	

[a]$P_{heterogeneity}$ (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks.
[b]Participants with cumulative mean BMI < 18.5 kg/m^2 were excluded.
[c]Adjusted for cumulative mean BMI, alcohol, folate, vitamin D, calcium, caloric and red meat intake, current smoking status, smoking before 30 years of age, current multivitamin use, current aspirin use, previous sigmoidoscopy, and family history of colorectal cancer.

our data as well as previous studies do not support this explanation.

Our study has limitations because of its observational design. First, among categories of BMI (or physical activity), there were differences in the distribution of additional exposures that might influence colorectal cancer risk, including smoking status, alcohol and folate intake, and aspirin use. Nonetheless, adjustment for these and additional known con-founders did not materially alter the results. Second, the fact that we were unable to obtain cancer tissue data from all colorectal cancer cases in the 2 cohorts represents another inherent limitation. Nevertheless, the current analysis revealed no substantial difference in the distribution of the exposures between patients with and without tumor tissue data. Third, we used only one tissue marker (nuclear CTNNB1) in the WNT pathway, and it is not a perfect measure of WNT pathway

Table 6. BMI, physical activity, and colorectal cancer risk according to nuclear CTNNB1 expression status in the combined cohort of men and women

	BMI[a] and physical activity categories					
	BMI <25 (low), MET-h/wk ≥9 (high activity)	BMI <25 (low), MET-h/wk <9 (low activity)	BMI ≥25 (high), MET-h/wk ≥9 (high activity)	BMI ≥25 (high), MET-h/wk <9 (low activity)	P_{trend}[b]	$P_{heterogeneity}$[c]
All colorectal cancers[a] (N = 767)						
Cases/person-years	250/753,448	96/348,917	246/581,931	175/370,200		
Multivariate HR[d] (95% CI)	1 (referent)	1.01 (0.79–1.28)	1.17 (0.97–1.40)	1.53 (1.25–1.87)	<0.0001	
CTNNB1 (−) colorectal cancers (N = 415)						
Cases/person-years	128/753,560	50/348,952	130/582,036	107/370,257		
Multivariate HR[d] (95% CI)	1 (referent)	0.99 (0.71–1.38)	1.22 (0.95–1.57)	1.82 (1.40–2.38)	<0.0001	0.05
CTNNB1 (+) colorectal cancers (N = 352)						
Cases/person-years	122/753,563	46/348,957	116/582,039	68/370,302		
Multivariate HR[d] (95% CI)	1 (referent)	1.03 (0.73–1.46)	1.10 (0.85–1.43)	1.21 (0.89–1.65)	0.22	

[a]Participants with cumulative mean BMI < 18.5 kg/m^2 were excluded.
[b]To obtain a P_{trend}, low-BMI/high-activity, low-BMI/low-activity, high-BMI/high-activity, and high-BMI/low-activity categories were coded as 0, 1, 1, and 2, respectively.
[c]$P_{heterogeneity}$ (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks.
[d]Adjusted for alcohol, folate, vitamin D, calcium, caloric and red meat intake, current smoking status, smoking before 30 years of age, current multivitamin use, current aspirin use, previous sigmoidoscopy, and family history of colorectal cancer.
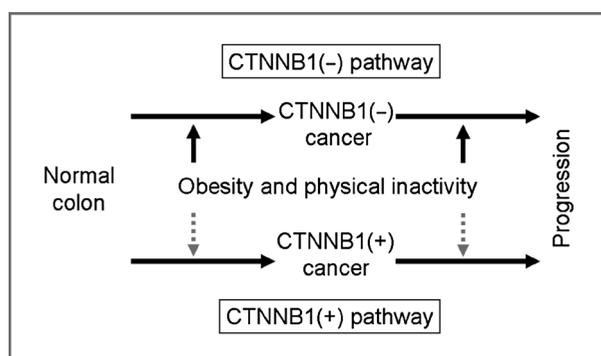
Figure 1. The putative relationship between obesity, physical inactivity, and colorectal tumor evolution pathways by cellular CTNNB1 status, based on the data by the current study and the previous study (17). The current study suggests that energy balance exposures (i.e., obesity and physical activity level) influence CTNNB1-negative colorectal cancer risk but not CTNNB1-positive cancer risk (left). Our previous data suggest that an energy balance exposure such as physical activity may influence progression of CTNNB1-negative tumor after colorectal cancer diagnosis (right; ref. 17). In contrast, progression of CTNNB1-positive tumors seemed to be independent of energy balance status of patients (17).

activation. Future studies using other WNT pathway-related markers will provide further insight into the influence of host energy balance status on specific tumorigenic pathways.

Our study possesses several key strengths. Prospective collection of anthropometric and lifestyle data over the 20-year period enabled evaluation of the long-term influence of BMI and physical activity, free from the potential for recall bias. Furthermore, the use of cumulative mean BMI and physical activity might decrease misclassification. We showed that analysis using the baseline BMI (to minimize potential reverse causation) yielded similar results, suggesting robustness of our findings. Our ability to control for potential confounding was strengthened as a result of low attrition rates in both studies, and the availability of exposure data from multiple time points during follow-up. Importantly, we took molecular pathologic epidemiology (MPE) approach (31, 32), which integrates analyses of epidemiologic exposures, tumor tissue biomarkers, and cancer risk based on the unique tumor principle (33, 34). MPE research has provided insights into tumor heterogeneity as well as the etiology and molecular pathogenesis of cancer (31–41). MPE paradigms have been evolving and adoption of the MPE concept has become worldwide (35–39, 42–50).

In conclusion, our current study suggests that increasing BMI is associated with a higher risk of CTNNB1-negative colorectal cancer but not with risk of CTNNB1-positive cancer subtype. In addition, physical activity level seems to decrease the risk of CTNNB1-negative colorectal cancer. These data suggest that energy balance status exerts its effect in a specific carcinogenic pathway that is less likely dependent on WNT/CTNNB1 activation. In the future, we may be able to identify individuals who are susceptible to the development of CTNNB1-negative tumors, and lifestyle preventive measures can be taken. Thus, our findings may help us develop better cancer prevention strategies.

Authors' Contributions

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