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

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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 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/m² increment; \(P_{\text{trend}} = 0.0001\)] but not with CTNNB1-positive cancer risk (multivariate HR = 1.07; 95% CI, 0.92–1.25 for 5.0 kg/m² increment; \(P_{\text{trend}} = 0.36\); \(P_{\text{heterogeneity}} = 0.027\), 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; \(P_{\text{trend}} = 0.044\)) but not with CTNNB1-positive cancer risk (multivariate HR = 0.98; 95% CI, 0.91–1.05 for 10 MET-h/wk increment; \(P_{\text{trend}} = 0.60\)). 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.
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 updated every 2 years. A validation study has been described previously (19). The concordance between the 2 observers was 0.90 (k = 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/m²), 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

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.

Ascertained 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 (k = 0.80; P < 0.0001), indicating substantial agreement.
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, ≥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, ≥30 kg/m²).

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/m² increment; P_{trend} = 0.01). Compared with men having BMI of 18.5–22.9 kg/m², multivariate HR for BMI of 27.5–29.9 kg/m², or BMI of ≥30 kg/m², 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/m² 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/m² 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/m² 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
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 colorectal cancer (multivariate HR = 1.34; 95% CI, 1.18–1.53, for a 5.0 kg/m² increment; P_trend < 0.0001). Compared with patients with BMI of 18.5–22.9 kg/m², multivariate HR for BMI of 27.5–29.9 kg/m², or BMI of ≥30 kg/m², 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/m² 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/m² 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/m² 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...
10 MET-h/wk increment; $P_{\text{trend}} = 0.044$). 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; $P_{\text{trend}} = 0.60$); however, the association between physical activity and the cancer risk did not significantly differ by tumor CTNNB1 status ($P_{\text{heterogeneity}} = 0.29$).

### 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

<table>
<thead>
<tr>
<th>Cumulative mean BMI</th>
<th>&lt;18.5</th>
<th>18.5–22.9</th>
<th>23.0–24.9</th>
<th>25.0–27.4</th>
<th>27.5–29.9</th>
<th>≥30</th>
<th>(P_{\text{trend}}^{a})</th>
<th>(P_{\text{heterogeneity}}^{b})</th>
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<tbody>
<tr>
<td>Men (HPFS) All colorectal cancers (.N = 368.)</td>
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<tr>
<td>Cases/person-years</td>
<td>0/1,740</td>
<td>54/133,142</td>
<td>99/215,324</td>
<td>121/23,950</td>
<td>60/110,178</td>
<td>34/67,656</td>
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<tr>
<td>Age-adjusted HR (95% CI)</td>
<td>1 (referent)</td>
<td>1.17 (0.83–1.63)</td>
<td>1.26 (0.91–1.75)</td>
<td>1.38 (0.95–2.00)</td>
<td>1.28 (0.83–1.99)</td>
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<td>0.13</td>
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<tr>
<td>Multivariate HR (95% CI)</td>
<td>1 (referent)</td>
<td>1.18 (0.84–1.65)</td>
<td>1.26 (0.91–1.75)</td>
<td>1.36 (0.93–1.98)</td>
<td>1.30 (0.83–2.03)</td>
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<td>0.14</td>
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<td>CTNNB1 ((\leq)) colorectal cancers (.N = 183.)</td>
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<tr>
<td>Cases/person-years</td>
<td>0/1,740</td>
<td>22/133,168</td>
<td>46/215,374</td>
<td>61/239,559</td>
<td>34/110,204</td>
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<tr>
<td>Age-adjusted HR (95% CI)</td>
<td>1 (referent)</td>
<td>1.38 (0.82–2.31)</td>
<td>1.65 (1.01–2.70)</td>
<td>1.93 (1.12–3.32)</td>
<td>1.96 (1.06–3.62)</td>
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<td>0.009</td>
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<tr>
<td>Multivariate HR (95% CI)</td>
<td>1 (referent)</td>
<td>1.38 (0.82–2.32)</td>
<td>1.65 (1.00–2.71)</td>
<td>1.88 (1.08–3.26)</td>
<td>2.00 (1.07–3.73)</td>
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<td>CTNNB1 ((&gt;)) colorectal cancers (.N = 185.)</td>
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<tr>
<td>Cases/person-years</td>
<td>0/1,740</td>
<td>32/133,159</td>
<td>53/215,368</td>
<td>60/239,561</td>
<td>26/110,205</td>
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<tr>
<td>Age-adjusted HR (95% CI)</td>
<td>1 (referent)</td>
<td>1.02 (0.66–1.59)</td>
<td>1.01 (0.65–1.55)</td>
<td>1.01 (0.60–1.70)</td>
<td>0.83 (0.43–1.60)</td>
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<tr>
<td>Multivariate HR (95% CI)</td>
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<td>1.04 (0.67–1.62)</td>
<td>1.01 (0.65–1.56)</td>
<td>1.00 (0.59–1.70)</td>
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<tr>
<td>Cases/person-years</td>
<td>5/23,116</td>
<td>133/68,671</td>
<td>100/38,709</td>
<td>107/330,693</td>
<td>64/190,232</td>
<td>84/230,096</td>
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<td>Age-adjusted HR (95% CI)</td>
<td>1.14 (0.46–2.78)</td>
<td>1.17 (0.90–1.51)</td>
<td>1.41 (1.09–1.82)</td>
<td>1.42 (1.06–1.92)</td>
<td>1.59 (1.21–2.09)</td>
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<td>0.0003</td>
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<td>Multivariate HR (95% CI)</td>
<td>1.18 (0.48–2.88)</td>
<td>1.13 (0.87–1.46)</td>
<td>1.36 (1.05–1.76)</td>
<td>1.38 (1.02–1.87)</td>
<td>1.55 (1.16–2.06)</td>
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<tr>
<td>Cases/person-years</td>
<td>2/23,120</td>
<td>72/686,767</td>
<td>56/387,134</td>
<td>60/330,735</td>
<td>43/190,247</td>
<td>51/230,126</td>
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<tr>
<td>Age-adjusted HR (95% CI)</td>
<td>0.82 (0.20–3.34)</td>
<td>1.20 (0.84–1.70)</td>
<td>1.45 (1.03–2.05)</td>
<td>1.76 (1.20–2.57)</td>
<td>1.76 (1.23–2.52)</td>
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<tr>
<td>Multivariate HR (95% CI)</td>
<td>0.84 (0.20–3.42)</td>
<td>1.16 (0.82–1.65)</td>
<td>1.41 (1.00–1.99)</td>
<td>1.70 (1.06–2.49)</td>
<td>1.72 (1.18–2.49)</td>
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<td>CTNNB1 ((&gt;)) colorectal cancers (.N = 209.)</td>
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<tr>
<td>Cases/person-years</td>
<td>3/23,116</td>
<td>61/686,779</td>
<td>44/387,146</td>
<td>47/330,748</td>
<td>21/190,275</td>
<td>33/230,137</td>
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<tr>
<td>Age-adjusted HR (95% CI)</td>
<td>1.55 (0.48–4.95)</td>
<td>1.13 (0.76–1.66)</td>
<td>1.35 (0.92–1.98)</td>
<td>1.03 (0.62–1.69)</td>
<td>1.38 (0.99–2.11)</td>
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<tr>
<td>Multivariate HR (95% CI)</td>
<td>1.62 (0.51–5.20)</td>
<td>1 (referent)</td>
<td>1.08 (0.73–1.60)</td>
<td>1.31 (0.89–1.92)</td>
<td>1.00 (0.60–1.65)</td>
<td>1.34 (0.87–2.07)</td>
<td></td>
<td>0.23</td>
</tr>
</tbody>
</table>

\(a\)Statistical test in participants with cumulative mean BMI \(\geq 18.5\) kg/m\(^2\).

\(b\)Statistical test for a multivariate linear trend between CTNNB1-positive and -negative cancer risks in participants with cumulative mean BMI \(\geq 18.5\) kg/m\(^2\).

\(c\)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

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Cases/person-years</th>
<th>Multivariate HRc (95% CI)</th>
<th>P\text{trend}</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5 kg/m²</td>
<td>187/92,234</td>
<td>0.96 (0.39–2.33)</td>
<td>0.81</td>
</tr>
<tr>
<td>18.5–22.9 kg/m²</td>
<td>1,17/0.95–1.43</td>
<td>1 (referent)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>23.0–24.9 kg/m²</td>
<td>1,34/1.09–1.63</td>
<td>1.17 (0.93–1.47)</td>
<td>0.36</td>
</tr>
<tr>
<td>25.0–27.4 kg/m²</td>
<td>1,34/1.09–1.63</td>
<td>1.17 (0.93–1.47)</td>
<td>0.36</td>
</tr>
<tr>
<td>≥27.5 kg/m²</td>
<td>1,84/1.34–2.53</td>
<td>1.52 (1.19–1.98)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*P\text{heterogeneity} (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks in participants with cumulative mean BMI <18.5 kg/m².*

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

Another possible explanation for our findings is that the WNT-CTNNB1 pathway may tend to be dysregulated 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 decreases in the expression of Ctnnb1 in the normal colonic mucosa (15). Therefore, the WNT-CTNNB1 pathway may tend to be dysregulated 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 decreases in the expression of Ctnnb1 in the normal colonic mucosa (15). Therefore,
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 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 5. Physical activity and colorectal cancer risk according to nuclear CTNNB1 expression status in the combined cohort of men and women

<table>
<thead>
<tr>
<th>Physical activity (MET-h/wk)</th>
<th>Cases/person-years</th>
<th>Multivariate HRc (95% CI)</th>
<th>P&lt;sub&gt;trend&lt;/sub&gt;</th>
<th>P&lt;sub&gt;heterogeneity&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3.0</td>
<td>92/255,551</td>
<td>1 (referent)</td>
<td>0.94 (0.73–1.22)</td>
<td>0.78 (0.58–1.04)</td>
</tr>
<tr>
<td>3.0–8.9</td>
<td>179/463,566</td>
<td>187/491,022</td>
<td>108/307,464</td>
<td>201/536,894</td>
</tr>
<tr>
<td>9.0–17.9</td>
<td>187/491,022</td>
<td>108/307,464</td>
<td>201/536,894</td>
<td></td>
</tr>
<tr>
<td>18.0–26.9</td>
<td>187/491,022</td>
<td>108/307,464</td>
<td>201/536,894</td>
<td></td>
</tr>
<tr>
<td>27.0/C21</td>
<td>201/536,894</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTNNB1 (−) colorectal cancers (N = 415)</td>
<td>58/255,580</td>
<td>99/463,629</td>
<td>101/491,100</td>
<td>102/536,978</td>
</tr>
<tr>
<td>Multivariate HR&lt;sup&gt;c&lt;/sup&gt; (95% CI)</td>
<td>1 (referent)</td>
<td>0.85 (0.61–1.18)</td>
<td>0.76 (0.55–1.06)</td>
<td>0.64 (0.44–0.94)</td>
</tr>
<tr>
<td>CTNNB1 (+) colorectal cancers (N = 352)</td>
<td>34/255,602</td>
<td>80/463,657</td>
<td>86/491,109</td>
<td>99/536,985</td>
</tr>
<tr>
<td>Multivariate HR&lt;sup&gt;c&lt;/sup&gt; (95% CI)</td>
<td>1 (referent)</td>
<td>1.11 (0.74–1.66)</td>
<td>1.08 (0.72–1.63)</td>
<td>1.01 (0.65–1.57)</td>
</tr>
</tbody>
</table>

<sup>a</sup> P<sub>heterogeneity</sub> (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks.

<sup>b</sup>Participants with cumulative mean BMI < 18.5 kg/m<sup>2</sup> were excluded.

<sup>c</sup>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.

<table>
<thead>
<tr>
<th>BMI&lt;sup&gt;a&lt;/sup&gt; and physical activity categories</th>
<th>Cases/person-years</th>
<th>Multivariate HR&lt;sup&gt;c&lt;/sup&gt; (95% CI)</th>
<th>P&lt;sub&gt;trend&lt;/sub&gt;</th>
<th>P&lt;sub&gt;heterogeneity&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;25 (low), MET-h/wk &gt;9 (high activity)</td>
<td>250/753,448</td>
<td>96/348,917</td>
<td>246/581,931</td>
<td>175/370,200</td>
</tr>
<tr>
<td>BMI &lt;25 (low), MET-h/wk &lt;9 (low activity)</td>
<td>1 (referent)</td>
<td>1.01 (0.79–1.28)</td>
<td>1.17 (0.97–1.40)</td>
<td>1.53 (1.25–1.87)</td>
</tr>
<tr>
<td>BMI ≥25 (high), MET-h/wk &gt;9 (high activity)</td>
<td>128/753,560</td>
<td>50/348,952</td>
<td>130/582,036</td>
<td>107/370,257</td>
</tr>
<tr>
<td>BMI ≥25 (high), MET-h/wk &lt;9 (low activity)</td>
<td>1 (referent)</td>
<td>0.99 (0.71–1.38)</td>
<td>1.22 (0.95–1.57)</td>
<td>1.82 (1.40–2.38)</td>
</tr>
<tr>
<td>CTNNB1 (−) colorectal cancers (N = 415)</td>
<td>122/753,563</td>
<td>46/348,957</td>
<td>116/582,039</td>
<td>68/370,302</td>
</tr>
<tr>
<td>CTNNB1 (+) colorectal cancers (N = 352)</td>
<td>1 (referent)</td>
<td>1.03 (0.73–1.46)</td>
<td>1.10 (0.85–1.43)</td>
<td>1.21 (0.89–1.65)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Participants with cumulative mean BMI < 18.5 kg/m<sup>2</sup> were excluded.

<sup>b</sup>To obtain a P<sub>trend</sub>, 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.

<sup>c</sup>P<sub>heterogeneity</sub> (for a multivariate linear trend) between CTNNB1-positive and -negative cancer risks.

<sup>d</sup>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.
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.

Disclosures of Potential Conflicts of Interest
No potential conflicts of interest were disclosed. The content is solely the responsibility of the authors and does not necessarily represent the official views of National Cancer Institute or NIH.

Authors' Contributions

References


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

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