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*Research Article*

# Physical Activity, Tumor PTGS2 Expression, and Survival in Patients with Colorectal Cancer

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## Abstract

**Background:** Higher levels of physical activity are associated with lower colorectal carcinoma incidence and mortality, perhaps through influencing energy balance, cellular prosta7 systemic inflammation. Although evidence suggests interactive effects of energetics, sedentary lifestyle, and tumor CTNNB1 (β-catenin) or CDKN1B (p27) status on colon cancer prognosis, interactive effects of physical activity and tumor PTGS2 (the official symbol for COX-2) status on clinical outcome remain unknown.

**Methods:** Using molecular pathological epidemiology database of 605 stage I–III colon and rectal cancers in two prospective cohort studies (the Nurse's Health Study and the Health Professionals Follow-up Study), we examined patient survival according to postdiagnosis physical activity and tumor PTGS2 status (with 382 PTGS2-positive and 223 PTGS2-negative tumors by immunohistochemistry). Cox proportional hazards models were used to calculate colorectal cancer-specific mortality HR, adjusting for clinical and other tumor variables including microsatellite instability status.

**Results:** Among PTGS2-positive cases, compared with the least active first quartile, the multivariate HRs (95% confidence interval) were 0.30 (0.14–0.62) for the second, 0.38 (0.20–0.71) for the third, and 0.18 (0.08–0.41) for the fourth quartile of physical activity level ($P_{trend} = 0.0002$). In contrast, among PTGS2-negative cases, physical activity level was not significantly associated with survival ($P_{trend} = 0.84$; $P_{interaction} = 0.024$, between physical activity and tumor PTGS2 status).

**Conclusions:** Postdiagnosis physical activity is associated with better survival among patients with PTGS2-positive tumors but not among patients with PTGS2-negative tumors.

**Impact:** Immunohistochemical PTGS2 expression in colorectal carcinoma may serve as a predictive biomarker in pathology practice, which may predict stronger benefit from exercise. *Cancer Epidemiol Biomarkers Prev; 22(6); 1142–52. ©2013 AACR.*

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## Introduction

Higher levels of physical activity are associated with lower risks of not only developing colorectal cancer (1–6) but also dying of the disease (7–19). Accumulating evidence suggests that the potential antineoplastic effect of physical activity may be mediated by decreased systemic inflammatory status (20), through a reduction in prostaglandin $E_2$ ($PGE_2$) synthesis (21–23).

PTGS2 (the official symbol for COX-2) and its enzymatic product, $PGE_2$, are key contributors to inflammatory responses and play important roles in colorectal cancer development and progression (24–27). Regular use of aspirin or nonsteroidal anti-inflammatory drugs (NSAID) has been associated with lower risks of colorectal cancer incidence and mortality, at least in part, through inhibition of PTGS2-related pathways (25, 28–31). Because physical activity may also modulate $PGE_2$ synthesis, we hypothesized that the association of physical activity with colorectal cancer survival might be stronger for patients with PTGS2-expressing tumors than for those with PTGS2-nonexpressing tumors.

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To test this hypothesis, we conducted a study of 605 patients with colorectal cancer within 2 prospective cohort studies in which we collected validated data on physical activity after diagnosis of colorectal cancer and also assessed status of tumor PTGS2 expression.

## Materials and Methods

### Study group

We used data from 2 prospective cohort studies: the Nurses' Health Study (NHS, $N = 121{,}701$ women followed since 1976), and the Health Professionals Follow-up Study (HPFS, $N = 51{,}529$ men followed since 1986; refs. 32, 33). Biennial questionnaires were used to collect data on dietary and lifestyle factors (including level of physical activity, aspirin use, smoking habits, and alcohol consumption) and family history of colorectal cancer. We also ascertained new cases of colorectal cancers. In total, 1,229 men in the HPFS and 3,580 women in the NHS were diagnosed as having colorectal cancer (up to 2006). We collected paraffin-embedded tissue blocks from hospitals where patients with colorectal cancer had undergone tumor resection. We also collected diagnostic biopsy specimens for patients with rectal cancer who had received preoperative treatment. Considering a continuum of pathological and molecular features from rectum to proximal colon (34, 35), we included both colon and rectal cancers in the current study. Tissue sections from all colorectal cancer cases were reviewed by a pathologist (S. Ogino), and the diagnosis confirmed. On the basis of the availability of tumor tissue data, postdiagnosis physical activity data, and follow-up data, a total of 605 colorectal cancer cases were included (Table 1). Within the cohort studies, there were no significant differences in demographic features between cases with available tumor tissue specimens and those without (28, 32). Informed consent was obtained from all study subjects. This study was approved by the Harvard School of Public Health and Brigham and Women's Hospital (Boston, MA) Institutional Review Boards.

### Assessment of physical activity

Leisure time physical activity was evaluated every 2 years in both cohorts, as previously described, and validated against subject diaries (36, 37). Subjects reported the duration of physical activity (ranging from 0 to 11 or more h/wk) engaged in walking (at usual pace), jogging, running, bicycling, swimming laps, racket sports, other aerobic exercises, lower intensity exercise (yoga, toning, stretching), or other vigorous activities (38). Each activity on the questionnaire was assigned a metabolic equivalent task (MET) score (38). MET scores for specific activities represent the activity-related metabolic rate divided and the resting metabolic rate (11, 32). In the present study, values for individual activities were summed to give a total MET-h/wk score. Because we observed differences in the distribution of reported physical activity levels between men and women, we classified physical activity level by generating sex-specific quartiles. To avoid assessment during the period of active oncologic treatment, the first assessment of physical activity was collected at least 1 year, but no more than 4 years after cancer diagnosis (median, 17 months; ref. 32). To minimize bias due to declining physical activity in the period around cancer recurrence or death, patients with known metastatic disease (stage IV) were excluded from this analysis, and physical activity was assessed at a single postdiagnosis time point (8, 32).

### Assessment of mortality

Ascertainment of deaths was accomplished by reporting from family members, or postal authorities (in the case of non-responders), and by searching for participants in the National Death Index (36). Following medical record review, the cause of death was assigned by study physicians (29, 36).

### Sequencing of *BRAF* and *KRAS* and microsatellite instability analysis

DNA was extracted from tumor tissue, and PCR and pyrosequencing targeted for *BRAF* (codon 600), and *KRAS* (codons 12 and 13), were conducted as previously described (39–41). MSI analysis was conducted by PCR using 10 microsatellite markers (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250, D18S55, D18S56, D18S67, and D18S487; ref. 41). MSI-high was defined as the presence of instability in $\geq$30% of the markers. Microsatellite instability (MSI)-low (<30% unstable markers) tumors were grouped with microsatellite stable (MSS) tumors (no unstable markers) because we have previously shown that these 2 groups show similar features (41).

### Methylation analyses for CpG islands and LINE-1

Using real-time PCR (MethyLight) on bisulfite-treated DNA, we quantified DNA methylation in 8 CIMP-specific promoters [*CACNA1G*, *CDKN2A* (*p16*), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*; refs. 41–43]. CIMP-high was defined as the presence of $\geq$6/8 methylated promoters, and CIMP-low/0 as 0/8-5/8 methylated promoters, according to established criteria (44). To quantify LINE-1 methylation, a pyrosequencing assay was used, as previously described (33, 45, 46).

### Immunohistochemical analyses

Immunohistochemical analysis methods for CDKN1B (p27; ref. 47), CTNNB1 (β-catenin; ref. 32), and PTGS2 (COX-2; ref. 28) expression have previously been described, using mouse anti-CDKN1B (Clone 57, BD Transduction Laboratories, Item No. 610242; dilution, 1:200), mouse anti-CTNNB1 (Clone 14, BD Transduction Laboratories, Item No. 610153; dilution, 1:400), and mouse anti-PTGS2 (Clone CX229, Cayman Chemical, Item No. 160112; dilution, 1:300). Appropriate positive and negative controls were included in each run of immunohistochemistry.

Each immunohistochemical marker was interpreted by a pathologist (CDKN1B and PTGS2 by S. Ogino; CTNNB1

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**Table 1.** Clinical, pathologic, and molecular characteristics of colorectal cancer cases according to postdiagnosis physical activity quartile

| Clinical, pathologic, or molecular feature | Total *N* | Postdiagnosis physical activity quartile | | | | *P* |
|---|---|---|---|---|---|---|
| | | Q1 (lowest) | Q2 (second) | Q3 (third) | Q4 (highest) | |
| All cases | 605 | 152 | 146 | 158 | 149 | |
| Sex | | | | | | 0.99 |
| Male (HPFS) | 305 (50%) | 77 (51%) | 75 (51%) | 78 (49%) | 75 (50%) | |
| Female (NHS) | 300 (50%) | 75 (49%) | 71 (49%) | 80 (51%) | 74 (50%) | |
| Mean age (SD) | 67.3 (8.0) | 68.2 (8.5) | 67.8 (8.1) | 66.7 (7.5) | 66.4 (7.6) | 0.025 |
| BMI (kg/m$^2$) | | | | | | 0.017 |
| <30 | 503 (83%) | 116 (76%) | 123 (84%) | 130 (82%) | 134 (90%) | |
| ≥30 | 102 (17%) | 36 (24%) | 23 (16%) | 28 (18%) | 15 (10%) | |
| Family history of colorectal cancer in first-degree relative(s) | | | | | | 0.93 |
| (−) | 482 (80%) | 122 (80%) | 114 (78%) | 128 (81%) | 118 (79%) | |
| (+) | 123 (20%) | 30 (20%) | 32 (22%) | 30 (19%) | 31 (21%) | |
| Year of diagnosis | | | | | | 0.54 |
| Before 1995 | 243 (40%) | 55 (36%) | 65 (45%) | 64 (41%) | 59 (40%) | |
| 1995 to 2006 | 362 (60%) | 97 (64%) | 81 (55%) | 94 (59%) | 90 (60%) | |
| Postdiagnosis aspirin use | | | | | | 0.80 |
| Nonuser | 368 (61%) | 93 (62%) | 85 (58%) | 95 (60%) | 95 (64%) | |
| Aspirin user | 236 (39%) | 58 (38%) | 61 (42%) | 63 (40%) | 54 (36%) | |
| Postdiagnosis smoking status | | | | | | 0.10 |
| Never | 238 (41%) | 60 (42%) | 58 (41%) | 57 (38%) | 63 (43%) | |
| Former | 305 (53%) | 72 (51%) | 68 (49%) | 84 (55%) | 81 (55%) | |
| Current | 37 (6.4%) | 10 (7.0%) | 14 (10%) | 11 (7.2%) | 2 (1.4%) | |
| Postdiagnosis alcohol consumption | | | | | | 0.85 |
| None | 229 (39%) | 58 (41%) | 56 (39%) | 62 (40%) | 53 (36%) | |
| Any | 362 (61%) | 85 (59%) | 88 (61%) | 94 (60%) | 95 (64%) | |
| Tumor location | | | | | | 0.92 |
| Cecum | 108 (18%) | 26 (17%) | 23 (16%) | 33 (21%) | 26 (17%) | |
| Ascending to transverse colon | 156 (26%) | 36 (24%) | 38 (26%) | 43 (28%) | 39 (26%) | |
| Splenic flexure to sigmoid | 190 (32%) | 47 (31%) | 50 (34%) | 44 (28%) | 49 (33%) | |
| Rectosigmoid and rectum | 149 (25%) | 43 (28%) | 35 (24%) | 36 (23%) | 35 (23%) | |
| Disease stage | | | | | | 0.99 |
| I | 161 (27%) | 38 (25%) | 38 (26%) | 43 (27%) | 42 (28%) | |
| II | 212 (35%) | 51 (34%) | 54 (37%) | 53 (34%) | 54 (36%) | |
| III | 165 (27%) | 45 (30%) | 38 (26%) | 45 (28%) | 37 (25%) | |
| Unknown | 67 (11%) | 18 (12%) | 16 (11%) | 17 (11%) | 16 (11%) | |
| Tumor differentiation | | | | | | 0.70 |
| Well-to-moderate | 556 (93%) | 142 (94%) | 136 (94%) | 140 (91%) | 138 (93%) | |
| Poor | 42 (7.0%) | 9 (6.0%) | 9 (6.2%) | 14 (9.1%) | 10 (6.8%) | |
| CIMP status | | | | | | 0.21 |
| CIMP-low/0 | 483 (84%) | 116 (82%) | 119 (84%) | 123 (81%) | 125 (89%) | |
| CIMP-high | 92 (16%) | 26 (18%) | 22 (16%) | 29 (19%) | 15 (11%) | |
| MSI status | | | | | | 0.37 |
| MSS | 482 (84%) | 119 (83%) | 118 (85%) | 122 (81%) | 123 (88%) | |
| MSI-high | 90 (16%) | 25 (17%) | 21 (15%) | 28 (19%) | 16 (12%) | |
| LINE-1 methylation level [mean (SD)] | 61.8 (9.5) | 61.4 (9.9) | 60.8 (10.0) | 62.2 (9.3) | 62.8 (8.9) | 0.12 |
| *BRAF* mutation | | | | | | 0.77 |
| (−) | 510 (89%) | 130 (90%) | 124 (89%) | 131 (87%) | 125 (90%) | |
| (+) | 64 (11%) | 14 (9.7%) | 16 (11%) | 20 (13%) | 14 (10%) | |
| *KRAS* mutation | | | | | | 0.25 |

*(Continued on the following page)*

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**Table 1.** Clinical, pathologic, and molecular characteristics of colorectal cancer cases according to postdiagnosis physical activity quartile (Cont'd)

| Clinical, pathologic, or molecular feature | Total *N* | Postdiagnosis physical activity quartile | | | | *P* |
|---|---|---|---|---|---|---|
| | | Q1 (lowest) | Q2 (second) | Q3 (third) | Q4 (highest) | |
| (−) | 364 (63%) | 94 (65%) | 87 (62%) | 104 (68%) | 79 (57%) | |
| (+) | 213 (37%) | 51 (35%) | 53 (38%) | 49 (32%) | 60 (43%) | |
| CDKN1B (p27) expression | | | | | | 0.36 |
| (−) | 234 (39%) | 52 (34%) | 64 (44%) | 63 (40%) | 55 (37%) | |
| (+) | 371 (61%) | 100 (66%) | 82 (56%) | 95 (60%) | 94 (63%) | |
| Nuclear CTNNB1 (β-catenin) expression | | | | | | 0.53 |
| (−) | 290 (53%) | 76 (58%) | 72 (54%) | 75 (53%) | 67 (49%) | |
| (+) | 255 (47%) | 56 (42%) | 62 (46%) | 66 (47%) | 71 (51%) | |
| PTGS2 (COX-2) expression | | | | | | 0.43 |
| (−) | 223 (37%) | 61 (40%) | 58 (40%) | 56 (35%) | 48 (32%) | |
| (+) | 382 (63%) | 91 (60%) | 88 (60%) | 102 (65%) | 101 (68%) | |

NOTE: (%) indicates the proportion of cases with a specific clinical, pathologic, or molecular feature in a given physical activity quartile. A $\chi^2$ *P* value is given for comparison across quartiles. ANOVA was used to compare the means of age and LINE-1 methylation. The Bonferroni-corrected *P* value for significance was $P = 0.0026$ (0.05/19).

by T. Morikawa) unaware of other data. For agreement studies, a random selection of more than 100 cases for each marker was examined by a second observer (CDKN1B by K. Shima; CTNNB1 by S. Ogino; PTGS2 by T. Morikawa) unaware of other data. The concordance between the 2 observers (all $P < 0.001$) was $\kappa = 0.60$ for CDKN1B, $\kappa = 0.80$ for CTNNB1, $\kappa = 0.69$ for PTGS2, indicating substantial agreement.

### Statistical analysis

For all statistical analyses, we used SAS software (Version 9.2, SAS Institute). All *P* values were 2-sided and statistical significance was set at a *P* value of 0.05. Our primary hypothesis was that the association of physical activity with survival differed by tumor PTGS2 status. Nonetheless, we interpreted results cautiously, according to the guidelines (48), given that the fundamental study design used subgroup analyses (in strata of PTGS2 status) to assess clinical outcomes. The subgroups defined and hypotheses tested in the current study were not planned analyses when the 2 cohort studies began; rather, our study comprised 5 *post hoc* subgroup analyses. To test for differences in the distribution of categorical data, the $\chi^2$ test was conducted. One-way ANOVA was used to compare mean age and mean LINE-1 methylation level. The statistical significance level for cross-sectional assessment of clinicopathologic and molecular associations was adjusted by Bonferroni correction to $P = 0.0026$ (=0.05/19), given multiple hypothesis testing.

Kaplan–Meier method and log-rank test were used for survival analyses. Patients were observed from the cancer diagnosis, until death or January 1, 2011, whichever came first. For colorectal cancer–specific mortality, deaths from other causes were censored. To control for confounding, we used multivariate Cox proportional hazards regression models. A multivariate model initially included sex, age at diagnosis (continuous), body mass index (BMI; <30 vs. ≥30 kg/m$^2$), family history of colorectal cancer in a first-degree relative (absent vs. present), year of diagnosis (continuous), postdiagnosis aspirin use (regular user vs. nonuser), postdiagnosis smoking status (never vs. former/current smokers), postdiagnosis alcohol consumption (none vs. any), tumor location (proximal vs. distal), tumor differentiation (well to moderate vs. poor), CIMP (low/0 vs. high), MSI (MSS vs. high), LINE-1 methylation (continuous), and *BRAF* and *KRAS* mutations. To minimize residual confounding, disease stage (I vs. II vs. III) was used as a stratifying variable using the "strata" option in the SAS "proc phreg" command. For cases with missing information in any of the categorical covariates [postdiagnosis aspirin use (0.2%), postdiagnosis smoking status (4.1%), postdiagnosis alcohol consumption (2.3%), tumor location (0.3%), tumor differentiation (1.2%), CIMP (5.0%), MSI (5.5%), *BRAF* (5.1%), and *KRAS* (4.6%)], we included those cases in the majority category of the given covariate. We confirmed that excluding cases with missing information in any of the covariates did not substantially alter results (data not shown). An interaction was assessed by the Wald test on interaction terms that were the cross-products of the variables of interest.

## Results

### Characteristics of colorectal cancer patients

Characteristics of the 605 participants with stage I–III colorectal cancer in the 2 prospective cohort studies are summarized according to postdiagnosis physical activity quartile in Table 1. Physically active individuals tended to be younger and leaner than physically inactive individuals.

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Among the 605 tumors, 382 (63%) were PTGS2-positive cases, whereas 223 (37%) were negative for PTGS2. Supplementary Table S1 summarizes characteristics of cases according to tumor PTGS2 expression status.

### Physical activity and survival of colorectal cancer patients

During follow-up [median, 11.9 (interquartile range, 7.9–15.5) years for censored cases], there were 253 deaths, including 89 colorectal cancer–specific deaths. We initially examined the relation between physical activity (quartiles) and patient survival in each cohort, separately (Table 2). Compared with participants who reported the lowest levels of postdiagnosis physical activity (first quartile, Q1), those reporting higher levels of physical activity experienced lower colorectal cancer–specific mortality in Kaplan–Meier analyses (log-rank $P = 0.0044$ among men in the HPFS and $P = 0.027$ among women in the NHS). In univariate and multivariate Cox regression analyses, compared with participants in the lowest quartile (Q1), higher levels of physical activity were associated with lower mortality in both men and women (Table 2). There was no significant interaction between postdiagnosis physical activity and sex/cohort ($P_{interaction} = 0.47$).

When men and women were combined, compared with participants in Q1, those reporting higher levels of physical activity (Q2–Q4) experienced lower colorectal cancer–specific mortality in Kaplan–Meier analysis (log-rank $P = 0.0002$; Fig. 1). In multivariate Cox regression analyses, compared with Q1, the multivariate HR was 0.42 [95% confidence interval (CI), 0.24–0.75] for Q2, 0.54 (95% CI, 0.32–0.91) for Q3, and 0.29 (95% CI, 0.15–0.56) for Q4 ($P_{trend} = 0.0006$; Table 2).

### Prognostic association of physical activity in strata of PTGS2 status

We examined the association between postdiagnosis physical activity quartile and patient survival in strata of tumor PTGS2 status. Notably, for PTGS2-positive cases, compared with the least active participants (Q1), those reporting higher levels of physical activity (Q2–Q4) experienced lower colorectal cancer–specific mortality in Kaplan–Meier analysis (log-rank $P < 0.0001$; Fig. 2). In multivariate Cox regression analyses, compared with Q1, the multivariate HR was 0.30 (95% CI, 0.14–0.62) for Q2, 0.38 (95% CI, 0.20–0.71) for Q3, and 0.18 (95% CI, 0.08–0.41) for Q4 ($P_{trend} = 0.0002$; Table 3). In contrast, for PTGS2-negative cases, there appeared to be no significant relationship between physical activity and mortality (Fig. 2 and Table 3). Furthermore, there was a statistically significant interaction between postdiagnosis physical activity quartile and tumor PTGS2 status ($P_{interaction} = 0.024$; Table 3).

In the analysis of overall mortality, the difference in the prognostic association of physical activity between PTGS2-positive and PTGS2-negative cases was somewhat attenuated (Fig. 2 and Table 3).

### Prognostic association of physical activity in strata of PTGS2 and other selected variables

In exploratory analyses, we examined the association between postdiagnosis physical activity quartile and patient survival stratified by tumor PTGS2 status and by other selected variables. Specifically, to establish that the association between postdiagnosis physical activity and survival in PTGS2-positive tumors was not attributable to differences in postdiagnosis aspirin use, we conducted an analysis limited to postdiagnosis aspirin nonusers and obtained results (Supplementary Table S2) consistent with the primary study findings (Table 3).

We previously reported that the association of postdiagnosis physical activity with cancer-specific survival was modified by tumor CDKN1B (49) and nuclear CTNNB1 status (32). Using physical activity quartile categories, we conducted analysis stratified by CDKN1B status (Supplementary Table S3) or nuclear CTNNB1 status (Supplementary Table S4). These analyses confirmed our prior associations between postdiagnosis physical activity and mortality among patients with CDKN1B-positive tumors or nuclear CTNNB1-negative tumors (32, 49).

## Discussion

We examined the hypothesis that the beneficial prognostic association of physical activity might be stronger in patients with PTGS2-positive colorectal cancer, compared with those with PTGS2-negative tumors. In stage I–III PTGS2-positive colorectal cancer, we found that postdiagnosis physical activity was associated with significantly better colorectal cancer–specific survival, whereas postdiagnosis physical activity was not significantly associated with survival among PTGS2-negative cases. These results provide evidence for an interactive effect of physical activity and tumor PTGS2 expression in determining tumor behavior and may give us clues to a role of energy balance in tumor progression and clinical outcome. In addition, tumor PTGS2 status may serve as a predictive biomarker of the beneficial effect of exercise, which can be recommended as part of a program of personalized health care.

Analysis of molecular biomarkers is increasingly important in colorectal and other cancers (50–71). Examining interactions between host factors and tumor markers has emerged as a promising study design in the evolving interdisciplinary field of molecular pathological epidemiology (MPE; refs. 72–75). As an integral part of a more expansive field of "Integrative Epidemiology" (76), MPE specifically addresses molecular and phenotypic heterogeneity of any given disease. MPE integrates molecular pathology and epidemiology to address interactive effects of lifestyle, genetic, and environmental factors and specific cellular molecular features on disease evolution and progression (72–75). MPE research may be clinically useful and can contribute to personalized medicine, as our current study suggests that tumor PTGS2 status may improve the

**Table 2.** Colorectal cancer mortality by postdiagnosis physical activity quartile

| Postdiagnosis physical activity quartile (MET-h/wk) | No. | Colorectal cancer–specific mortality | | | | Overall mortality | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of events | Univariate HR (95% CI) | Stage-stratified HR (95% CI) | Multivariate stage-stratified HR[a] (95% CI) | No. of events | Univariate HR (95% CI) | Stage-stratified HR (95% CI) | Multivariate stage-stratified HR[a] (95% CI) |
| Male | | | | | | | | | |
| Q1 (<6.4) | 77 | 17 | 1 (referent) | 1 (referent) | 1 (referent) | 42 | 1 (referent) | 1 (referent) | 1 (referent) |
| Q2 (6.4–18.4) | 75 | 8 | 0.42 (0.18–0.98) | 0.43 (0.19–1.00) | 0.38 (0.16–0.90) | 39 | 0.87 (0.56–1.34) | 0.87 (0.56–1.36) | 0.80 (0.52–1.25) |
| Q3 (18.6–46.5) | 78 | 13 | 0.64 (0.31–1.31) | 0.61 (0.30–1.27) | 0.69 (0.33–1.44) | 31 | 0.58 (0.36–0.92) | 0.59 (0.37–0.94) | 0.66 (0.41–1.06) |
| Q4 (≥47.1) | 75 | 3 | 0.15 (0.04–0.51) | 0.16 (0.05–0.53) | 0.17 (0.05–0.57) | 30 | 0.60 (0.38–0.96) | 0.62 (0.38–0.99) | 0.63 (0.39–1.02) |
| $P_{trend}$[b] | | | 0.0047 | 0.0051 | 0.0099 | | 0.035 | 0.044 | 0.086 |
| Female | | | | | | | | | |
| Q1 (<2.4) | 75 | 20 | 1 (referent) | 1 (referent) | 1 (referent) | 36 | 1 (referent) | 1 (referent) | 1 (referent) |
| Q2 (2.4–7.5) | 71 | 9 | 0.44 (0.20–0.96) | 0.46 (0.21–1.01) | 0.43 (0.19–0.94) | 25 | 0.64 (0.38–1.06) | 0.63 (0.38–1.06) | 0.66 (0.39–1.10) |
| Q3 (7.7–17.7) | 80 | 10 | 0.43 (0.20–0.91) | 0.48 (0.22–1.04) | 0.48 (0.22–1.04) | 27 | 0.60 (0.36–0.99) | 0.64 (0.39–1.06) | 0.64 (0.39–1.06) |
| Q4 (≥18.3) | 74 | 9 | 0.41 (0.19–0.90) | 0.42 (0.19–0.93) | 0.40 (0.18–0.89) | 23 | 0.53 (0.31–0.89) | 0.52 (0.31–0.89) | 0.56 (0.33–0.96) |
| $P_{trend}$[b] | | | 0.11 | 0.12 | 0.10 | | 0.064 | 0.064 | 0.10 |
| Combined | | | | | | | | | |
| Q1 | 152 | 37 | 1 (referent) | 1 (referent) | 1 (referent) | 78 | 1 (referent) | 1 (referent) | 1 (referent) |
| Q2 | 146 | 17 | 0.43 (0.24–0.76) | 0.45 (0.25–0.79) | 0.42 (0.24–0.75) | 64 | 0.76 (0.55–1.06) | 0.77 (0.55–1.08) | 0.76 (0.54–1.06) |
| Q3 | 158 | 23 | 0.52 (0.31–0.88) | 0.52 (0.31–0.88) | 0.54 (0.32–0.91) | 58 | 0.59 (0.42–0.83) | 0.59 (0.42–0.83) | 0.62 (0.44–0.88) |
| Q4 | 149 | 12 | 0.29 (0.15–0.55) | 0.30 (0.16–0.57) | 0.29 (0.15–0.56) | 53 | 0.57 (0.40–0.80) | 0.57 (0.40–0.81) | 0.61 (0.43–0.87) |
| $P_{trend}$[b] | | | 0.0011 | 0.0013 | 0.0006 | | 0.045 | 0.057 | 0.022 |
| $P_{interaction}$[c] | | | 0.58 | 0.50 | 0.47 | | 0.92 | 0.88 | 0.87 |

[a]The multivariate, stage-stratified Cox regression model initially included sex, age, BMI, family history of colorectal cancer in any first-degree relative, year of diagnosis, postdiagnosis aspirin use, postdiagnosis smoking status, postdiagnosis alcohol consumption, tumor location, tumor differentiation, CpG island methylator phenotype, microsatellite instability, LINE-1 methylation, and *BRAF* and *KRAS* mutations. A backward elimination with threshold of $P = 0.05$ was used to select variables in the final models.

[b]Tests for linear trend across categories were calculated by using the median value for each quartile of physical activity (MET-h/wk) as a continuous variable in a proportional hazards model.

[c]$P_{interaction}$ between physical activity quartile and sex/cohort.

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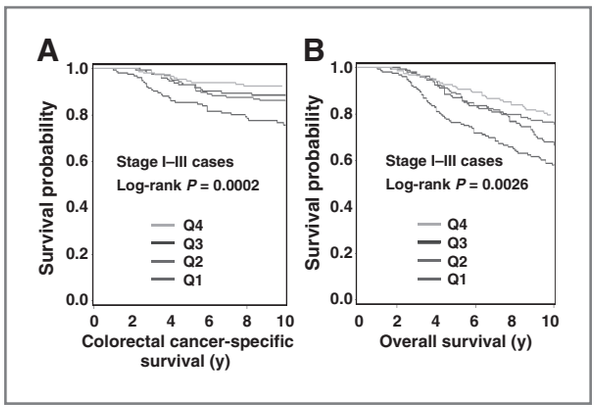


Figure 1. Kaplan–Meier curves for stage I–III patients with colorectal cancer. A, colorectal cancer–specific survival according to postdiagnosis physical activity quartile. B, overall survival according to postdiagnosis physical activity quartile.

identification of patients who will benefit most from physical activity.

Prospective observational data suggest that physically active colorectal cancer survivors have lower rates of cancer recurrence and death, compared with physically inactive survivors (7, 8, 10–19). Physical activity is a modifiable lifestyle factor, and thus its beneficial effect on cancer survival has considerable clinical implications (77–81). Identifying predictive biomarkers for clinical interventions is important in cancer research. As with any other oncologic interventions, it is unlikely patients will uniformly derive benefits from exercise, and it would be of great value to be able to identify patient characteristics or tumor molecular features that can predict response to lifestyle interventions. Molecular features of a primary tumor might be different from those of a corresponding recurrent/metastatic tumor. Nonetheless, tumor molecular features have been shown to be generally similar between primary and metastatic tumors (82, 83), and most tumor biomarkers rely on analyses of primary tumor tissues.

Several mechanisms have been postulated to underlie the influence of physical activity on colorectal cancer behavior, including decreased $PGE_2$ activity, reduced gut transit time and attenuation of hyperinsulinemia (21–23, 84–88). We have previously shown that physical activity appears to be more beneficial in patients with certain subtypes of colorectal cancers, including CTNNB1-negative tumors (32) and CDKN1B (p27)-expressing tumors (49). Nonetheless, colorectal cancer represents a group of complex diseases (89) and additional tumor biomarkers need to be explored. Our current findings suggest a possible effect of postdiagnosis physical activity in attenuating the aggressiveness of PTGS2-positive tumors. In addition, our exploratory data suggest that the beneficial association of postdiagnosis physical activity with colorectal cancer survival is not caused by postdiagnosis aspirin use. Postdiagnosis physical activity and aspirin use may act synergistically to attenuate tumor aggressiveness in patients with PTGS2-positive colorectal cancer. These findings are compatible with our hypothesis that physical activity may improve survival by inhibiting PTGS2 downstream effectors, such as $PGE_2$.

Interestingly, our data imply that even a modest amount of exercise (≥6.4 MET-h/wk in men and ≥2.4 MET-h/wk in women) significantly improves colorectal cancer–specific survival among patients with PTGS2-positive tumors. In the previous report (8, 9, 11, 14, 32), the beneficial effects of postdiagnosis physical activity on colorectal cancer survival were apparent in individuals who engaged in much higher levels of exercise. Therefore, our current data may help motivate inactive colorectal cancer survivors to engage in even modest levels of exercise. This apparent discrepancy might be in part because, unlike our current MPE study, the previous studies (8, 9, 11, 14) regarded all colorectal cancer cases (regardless of PTGS2 expression status) as a single disease entity without much consideration of heterogeneity in colorectal cancer biology between cases.

There are some limitations in this study including limited data on cancer treatment. Nonetheless, it is unlikely that chemotherapy use substantially differed according

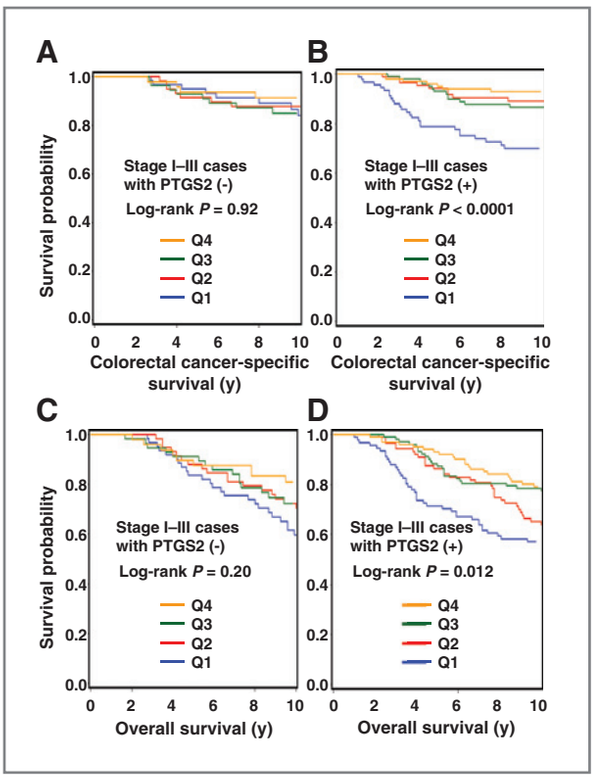


Figure 2. Kaplan–Meier curves for stage I–III colorectal cancer, stratified by tumor PTGS2 status. A, colorectal cancer–specific survival according to postdiagnosis physical activity quartile in PTGS2-negative cases. B, colorectal cancer–specific survival according to postdiagnosis physical activity quartile in PTGS2-positive cases. C, overall survival according to postdiagnosis physical activity quartile in PTGS2-negative cases. D, overall survival according to post-diagnosis physical activity quartile in PTGS2-positive cases.

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**Table 3.** Colorectal cancer mortality by postdiagnosis physical activity quartile, stratified by PTGS2 (COX-2) status

| Postdiagnosis physical activity quartile | No. | Colorectal cancer–specific mortality | | | | Overall mortality | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of events | Univariate HR (95% CI) | Stage-stratified HR (95% CI) | Multivariate stage-stratified HR[a] (95% CI) | No. of events | Univariate HR (95% CI) | Stage-stratified HR (95% CI) | Multivariate stage-stratified HR[a] (95% CI) |
| PTGS2 (COX-2) (−) | | | | | | | | | |
| Q1 | 61 | 8 | 1 (referent) | 1 (referent) | 1 (referent) | 28 | 1 (referent) | 1 (referent) | 1 (referent) |
| Q2 | 58 | 7 | 0.87 (0.32–2.41) | 0.96 (0.34–2.68) | 0.89 (0.32–2.51) | 24 | 0.83 (0.48–1.43) | 0.86 (0.49–1.49) | 0.87 (0.50–1.52) |
| Q3 | 56 | 8 | 1.07 (0.40–2.85) | 1.10 (0.41–2.94) | 1.14 (0.42–3.08) | 17 | 0.65 (0.36–1.20) | 0.67 (0.37–1.23) | 0.68 (0.37–1.26) |
| Q4 | 48 | 5 | 0.74 (0.24–2.27) | 0.82 (0.26–2.55) | 0.85 (0.27–2.67) | 13 | 0.52 (0.27–1.00) | 0.54 (0.28–1.05) | 0.65 (0.33–1.29) |
| $P_{trend}$[b] | | | 0.67 | 0.83 | 0.84 | | 0.18 | 0.27 | 0.40 |
| PTGS2 (COX-2) (+) | | | | | | | | | |
| Q1 | 91 | 29 | 1 (referent) | 1 (referent) | 1 (referent) | 50 | 1 (referent) | 1 (referent) | 1 (referent) |
| Q2 | 88 | 10 | 0.31 (0.15–0.63) | 0.29 (0.14–0.60) | 0.30 (0.14–0.62) | 40 | 0.72 (0.47–1.09) | 0.71 (0.46–1.08) | 0.70 (0.46–1.06) |
| Q3 | 102 | 15 | 0.38 (0.20–0.71) | 0.36 (0.19–0.67) | 0.38 (0.20–0.71) | 41 | 0.54 (0.36–0.82) | 0.54 (0.36–0.82) | 0.60 (0.39–0.91) |
| Q4 | 101 | 7 | 0.18 (0.08–0.41) | 0.18 (0.08–0.41) | 0.18 (0.08–0.41) | 40 | 0.57 (0.37–0.86) | 0.57 (0.37–0.86) | 0.57 (0.38–0.88) |
| $P_{trend}$[b] | | | 0.0004 | 0.0004 | 0.0002 | | 0.095 | 0.10 | 0.030 |
| $P_{interaction}$[c] | | | 0.040 | 0.030 | 0.024 | | 0.77 | 0.84 | 0.82 |

[a]The multivariate, stage-stratified Cox regression model included the same set of covariates selected as in Table 2.

[b]Tests for linear trend across categories were calculated by using the median value for each quartile of physical activity (MET-h/wk) as a continuous variable in a proportional hazards model.

[c]$P_{interaction}$ between physical activity quartile and tumor PTGS2 status.

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to tumor PTGS2 status, as this information was unavailable to physicians. In addition, our survival analyses were adjusted for cancer stage, on which treatment decisions are mainly based. Another limitation is that data on cancer recurrence were unavailable. Nonetheless, colorectal cancer–specific mortality was a reasonable surrogate for colorectal cancer–specific outcomes given the long follow-up of those who were censored. We limited our analysis to stage I–III disease for which a vast majority of patients could undergo potentially curative cancer resection and could exercise after recovery from surgery. Thus, it is likely that reverse causation may not be the only explanation for the apparent interactive effect of tumor PTGS2 status and physical activity.

There are advantages in using the data from the 2 U.S. nationwide prospective cohort studies. Data on anthropometric measurements (such as BMI), cancer staging, and other clinical, pathologic, and tumor molecular variables had been prospectively collected, blinded to patient survival. Cohort participants who were diagnosed with cancer were treated at hospitals throughout the United States and are thus more representative of colorectal cancer cases in the general Caucasian population than patients selected from a few academic hospitals. In addition, the comprehensive tumor tissue data enabled us to conduct MPE research (72–75) and assess the interaction between physical activity and tumor PTGS2 status.

In conclusion, our data provide evidence for a possible interactive effect of postdiagnosis physical activity and tumor PTGS2 expression status on colorectal cancer prognosis. Notably, the association between better survival and physical activity was observed only in participants with PTGS2-positive colorectal cancers, whereas no prognostic association was observed for physical activity in PTGS2-negative cases. Our findings not only give insight into the biology of colorectal cancer progression, adding to the expanding literature on energetics and inflammation but also have the potential to influence clinical recommendations relating to lifestyle modification after a diagnosis of colorectal cancer. Further studies are necessary to confirm our findings and to elucidate mechanisms that underlie the complex interactions between host energetics, inflammation, and tumor evolution and progression.

## Disclosure of Potential Conflicts of Interest

A.T. Chan was previously a consultant for Bayer Healthcare, Millennium Pharmaceuticals, and Pfizer Inc. This study was not funded by Bayer Healthcare, Millennium Pharmaceuticals, or Pfizer Inc. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

All of the authors revised the article critically for important intellectual content and approved the final version of the manuscript submitted for publication.

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**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** Z.R. Qian, T. Morikawa, C.S. Fuchs, A.T. Chan, S. Ogino
**Study supervision:** C.S. Fuchs, A.T. Chan, S. Ogino

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