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Article

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Physical Activity and Colorectal Cancer Prognosis According to Tumor-Infiltrating T Cells

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Abstract

Background: Evidence suggests that high-level physical activity may potentially reduce cancer mortality through its immune enhancement effect. We therefore hypothesized that survival benefits associated with physical activity might be stronger in colorectal carcinomas with lower immune reaction at diagnosis.

Methods: Using molecular pathological epidemiology databases of 470 colon and rectal carcinoma cases in the Nurses' Health Study and the Health Professionals Follow-up Study, we assessed the prognostic association of postdiagnosis physical activity in strata of densities of $CD3^+$ cells, $CD8^+$ cells, CD45RO (PTPRC)$^+$ cells, or $FOXP3^+$ cells in tumor tissue. Cox proportional hazards regression model was used to adjust for potential confounders, including microsatellite instability, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation, *KRAS*, *BRAF*, and *PIK3CA* mutations, and expression of CTNNB1 (beta-catenin), PTGS2 (cyclooxygenase-2), and *IRS1*.

Results: The association of postdiagnosis physical activity with colorectal cancer-specific mortality differed by $CD3^+$ cell density ($P_{interaction} < .001$). Multivariable-adjusted colorectal cancer-specific mortality hazard ratios for a quartile-unit increase in physical activity were 0.56 (95% confidence interval = 0.38 to 0.83) among cases with the lowest quartile of $CD3^+$ cell density compared with 1.14 (95% confidence interval = 0.79 to 1.65) in cases with the highest quartile. We observed no differential survival association of physical activity by densities of $CD8^+$ cells, $CD45RO^+$ cells, or $FOXP3^+$ cells.

Conclusions: The association between postdiagnosis physical activity and colorectal cancer survival appeared stronger for carcinomas with lower T cell infiltrates, suggesting an interactive effect of exercise and immunity on colorectal cancer progression.

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Innate and adaptive immunity play crucial roles in suppressing tumor progression (1,2). In colorectal carcinomas, high-level infiltrates of $CD3^+$ cells, $CD8^+$ cells, and CD45RO $(PTPRC)^+$ cells in the tumor microenvironment have been associated with longer patient survival (3–7). Emerging evidence indicates that immunotherapies targeting immune checkpoint molecules such as *PDCD1* (programmed cell death 1, PD-1) and *CD274* (*PDCD1* ligand 1, PD-L1) can be effective in treating several cancer types (8), including colorectal cancer with high-level microsatellite instability (MSI) (9,10). Elucidating the interplay between tumor cells and the immune system is of considerable importance to further improve the efficacy of immunoprevention and immunotherapy strategies for cancer (11–17).

High-level physical activity has been associated with lower incidence and mortality of colorectal cancer (18–28). Evidence suggests that physical activity may prolong colorectal cancer patient survival through decreasing chronic inflammation in the tumor microenvironment and enhancing the T cell-mediated antitumor immune response (29–32). Colorectal cancer consists of a heterogeneous group of neoplasms due to complex interactions with environmental factors, host immune cells, and transformed cells (1,33). We considered that tumors that had progressed despite the presence of higher T cell reaction might have developed mechanisms to escape immune surveillance; such tumors with higher immune reaction might exhibit refractoriness to immunomodulatory effects of physical activity after cancer diagnosis. We therefore hypothesized that survival benefits associated with increased physical activity levels might be stronger for tumors with lower T cell reaction than for tumors with a higher reaction.

To test our hypothesis, we used a molecular pathological epidemiology database derived from two large prospective cohort studies in the United States with data on physical activity levels after colorectal cancer diagnosis, tumor molecular and immune features, and survival outcomes. We examined the interactive prognostic association of postdiagnosis physical activity levels and tumor-infiltrating T cells.

Methods

Study Population

We examined data from two large prospective cohort studies in the United States: the Nurses' Health Study (NHS, 121 701 women ages 30–55 years followed since 1976) and the Health Professionals Follow-up Study (HPFS, 51 529 men ages 40 to 75 years followed since 1986) (34). Study participants have been sent follow-up questionnaires biennially to update information on demographics, lifestyle factors, and medical history and to report newly diagnosed diseases including colorectal cancer. The follow-up rate has been more than 90% for each questionnaire cycle in both cohorts. The National Death Index was used to ascertain deaths of study participants and identify patients with unreported lethal colorectal cancer. Informed consent was obtained from all participants at study enrollment. This study was approved by the Human Subjects Committees at Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital (Boston, MA).

We included 470 colorectal cancer cases with available data on postdiagnosis physical activity levels and T cell densities in tumor tissue among participants diagnosed with colorectal cancer through 2008. We included both colon and rectal carcinoma cases based on the colorectal continuum model (35). Patients were followed until death or end of follow-up (June 30, 2014 for the NHS; January 1, 2014 for the HPFS), whichever came first. Study physicians, blinded to exposure data, reviewed medical records of patients diagnosed with colorectal cancer to collect data on tumor characteristics and to identify causes of death for deceased patients. We collected formalin-fixed paraffin-embedded tumor tissue samples from hospitals throughout the United States where participants with colorectal carcinoma underwent tumor resection. A single pathologist (SO), blinded to other data, conducted a central review of hematoxylin and eosin-stained tissue sections of all colorectal carcinoma cases and collected data on histopathological characteristics, including tumor differentiation and four lymphocytic reaction patterns (Crohn-like lymphoid reaction, peritumoral lymphocytic reaction, intratumoral periglandular reaction, and tumor-infiltrating lymphocytes) (36).

Assessment of Physical Activity Levels

In the NHS and HPFS, leisure-time physical activity has been assessed biennially since 1986 using a self-administered physical activity questionnaire, which was validated against physical activity diaries in which the participants documented the duration of time spent in activities as previously described (37). Participants reported the duration of physical activity for each component of physical activity. Based on this information, we calculated a metabolic equivalent task score (METS) (38), which was defined as the ratio of the metabolic rate of specific activities to the resting metabolic rate (39,40). We summed up the METS for each activity to obtain the total METS-hours/week. To avoid the period of active anti-cancer treatment, we used questionnaire data reported between 6 and 48 months after diagnosis of colorectal cancer. To minimize the bias arising from reduced physical activity due to the progression of disease, physical activity was evaluated at the earliest time period after the diagnosis of colorectal cancer. Taking into account sex differences in lifestyle and physical activity, we classified physical activity levels into sex-specific quartiles (39,40).

Immunohistochemistry

We constructed tissue microarrays from colorectal cancer tissue blocks (41) and conducted immunohistochemistry for CD3, CD8, CD45RO, and FOXP3, as previously described (7). We used an automated scanning microscope and the Ariol image analysis system (Genetix, CA) to measure T cell densities (cells/mm^2) in tumor tissue. We assessed up to four tissue microarray cores from each tumor and calculated the average density of each tumor-infiltrating T cell subset. If applicable, we categorized T cell densities into low vs high by the median value.

Immunohistochemical analyses for CTNNB1 (beta-catenin) expression (42), PTGS2 (cyclooxygenase-2) expression (39), and *IRS1* expression (40) were performed using a mouse anti-CTNNB1 antibody (BD Transduction Laboratories, CA), anti-PTGS2 antibody (Cayman Chemical, MI), and anti-IRS1 antibody (Millipore, Billerica, MA), respectively.

Analyses of Microsatellite Instability, DNA Methylation, and *KRAS*, *BRAF*, and *PIK3CA* Mutations

DNA was extracted from archival formalin-fixed, paraffin-embedded tumor tissue. We determined MSI status using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487), as previously described (43). We defined MSI-high as the presence of instability in 30% or more of the markers, and non-MSI-high as instability in less than 30% of the markers. Using bisulfite-treated DNA, we quantified DNA methylation in eight CpG island methylator phenotype (CIMP)-specific promoters (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*) and in long interspersed nucleotide element-1 (LINE-1) (44,45). We defined CIMP-high as the presence of six or more methylated promoters and CIMP-low/negative as five or fewer methylated promoters (45). We conducted polymerase chain reaction and pyrosequencing targeted for *KRAS* (codons 12, 13, 61, and 146) (46), *BRAF* (codon 600) (43), and *PIK3CA* (exons 9 and 20) (47).

Statistical Analysis

Detailed information on statistical methods is included in Supplementary Methods (available online). All statistical analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC), and all *P* values were two-sided. Our primary hypothesis testing was assessment of a statistical interaction (using the Wald test on the cross-product) between postdiagnosis physical activity levels (the median value of each decile category) and T cell densities in tumor tissue (the median value of each decile category) in the Cox proportional hazards regression model for colorectal cancer-specific mortality analysis. Variables for physical activity and T cell densities were treated as decile categorical variables to reduce the influential effect of a few arbitrary cutoff points. In our primary hypothesis testing on new discoveries, we used the α level of 0.005 (48). All other analyses including evaluations of stratum-specific hazard ratios (HRs) and survival curves represented secondary analyses. In our secondary and other exploratory analyses, we recognized multiple comparisons associated with those analyses and used the α level of 0.005. Outcome endpoints were colorectal cancer-specific mortality and overall mortality. Survival time was defined as the time since colorectal cancer diagnosis to death or the end of follow-up, whichever came first, and was left-truncated at the time of the first postdiagnosis questionnaire return.

To reduce bias due to the availability of postdiagnosis questionnaire data, the inverse probability weighting (IPW) method was used in all survival analyses (49–51). We estimated the probability of questionnaire return after colorectal cancer diagnosis using the multivariable logistic regression model as previously described (49) and used the inverse probability to weight each patient. When we performed sex-stratified IPW-adjusted Cox regression analyses without truncation of weight, the results remained consistent (data not shown). Multivariable sex-stratified Cox proportional hazards models initially included age at diagnosis, year of diagnosis, prediagnosis physical activity, postdiagnosis body mass index, history of colorectal cancer in any first-degree relatives, tumor location, tumor differentiation, disease stage, MSI status, CIMP, LINE-1 methylation level, *BRAF* mutation, *KRAS* mutation, *PIK3CA* mutation, nuclear CTNNB1 expression, PTGS2 expression, and *IRS1* expression. A backward elimination was performed with a threshold of *P* equals .05 to select variables for the final models. We also estimated HRs for a quartile-unit increase of postdiagnosis physical activity levels in strata of levels of T cell densities using a re-parameterization of the interaction term in a single regression model (45). The cases with missing data were included in the majority category of a given categorical covariate to limit the degrees of freedom of the models. For cases with missing data on LINE-1 methylation level (2.1%) and *IRS1* expression (12.0%), we assigned a separate indicator variable for each variable. We confirmed that excluding cases with missing information in any of the covariates did not alter our results substantially (data not shown). The proportionality of hazards assumption was evaluated using a time-dependent variable, which was the cross-product of the postdiagnosis physical activity variable and survival time ($P > .05$). Results of Cox regression analyses without IPW, which were similar to those with IPW, are shown in Supplementary Table 1 (available online). Survival probabilities were estimated using the IPW-adjusted Kaplan-Meier method and compared using the weighted log-rank test (52).

Results

We included 470 colorectal cancer cases with available data on postdiagnosis physical activity levels and T cell densities in tumor tissue. Table 1 summarizes the clinical, pathological, and molecular characteristics of colorectal cancer cases according to quartiles of postdiagnosis physical activity levels. During the median follow-up time of 17.3 years (interquartile range = 14.9 to 20.6 years) for all censored cases, there were 275 deaths from any cause, including 100 colorectal cancer-specific deaths. Postdiagnosis physical activity levels were associated with colorectal cancer-specific mortality overall (Table 2).

In our primary hypothesis testing, the association of postdiagnosis physical activity levels and colorectal cancer-specific mortality differed by $CD3^+$ cell density ($P_{interaction} < .001$; with the α level of 0.005; Table 2 and Supplementary Table 2, available online). The multivariable-adjusted HRs of colorectal cancer-specific mortality for a quartile-unit increase in postdiagnosis physical activity levels were 0.56 (95% confidence interval [CI] = 0.38 to 0.83) in the lowest quartile of $CD3^+$ cell density and 1.14 (95% CI = 0.79 to 1.65) in the highest quartile of $CD3^+$ cell density. The differential prognostic association was similarly observed in women and men, although statistical power was limited in each subgroup (Table 3). Figure 1 shows IPW-adjusted Kaplan-Meier survival curves of colorectal cancer-specific survival according to tertiles of postdiagnosis physical activity levels, and Table 4 shows HRs of cancer-specific mortality in each category of postdiagnosis physical activity levels. Taking into account the small number of events in each category, we used tertiles of postdiagnosis physical activity levels for these analyses. Considering the influence of arbitrary cutoff points, we entered postdiagnosis physical activity levels as a continuous variable into the models and obtained similar results (Supplementary Table 3, available online). When we excluded stage IV patients (Supplementary Table 4, available online) or patients who died within 6 months of the first postdiagnosis questionnaire return (n = 12, data not shown), we observed a similar interactive prognostic association of postdiagnosis physical activity levels and $CD3^+$ cell density ($P_{interaction} < .001$). We did not observe any statistically significant interaction of postdiagnosis physical activity levels with densities of $CD8^+$ cells, $CD45RO^+$ cells, or $FOXP3^+$ cells ($P_{interaction} > .13$; Table 2).

Table 1. Clinical, pathological, and molecular characteristics of colorectal cancer cases according to postdiagnosis physical activity levels

Characteristic*	All cases (n = 470)	Postdiagnosis physical activity levels			
		Quartile 1 (Lowest) (n = 114)	Quartile 2 (n = 121)	Quartile 3 (n = 117)	Quartile 4 (Highest) (n = 118)
Postdiagnosis physical activity levels (METS-h/wk), median (range)					
Female (n = 252, NHS)	7.3 (0–157.9)	0.7 (0–2.2)	3.7 (2.3–7.2)	10.2 (7.3–16.9)	36.0 (17.0–157.9)
Male (n = 218, HPFS)	15.4 (0–155.9)	1.0 (0–4.9)	8.6 (5.0–15.3)	23.0 (15.4–34.9)	57.3 (35.0–155.9)
Mean age ± SD, y	67.8 ± 8.0	69.4 ± 8.7	68.3 ± 7.9	67.0 ± 8.0	66.5 ± 7.2
Year of diagnosis					
1995 or before	209 (44%)	41 (36%)	61 (50%)	50 (43%)	57 (48%)
1996 to 2000	194 (41%)	56 (49%)	45 (37%)	47 (40%)	46 (39%)
2001 to 2008	67 (14%)	17 (15%)	15 (12%)	20 (17%)	15 (13%)
Family history of colorectal cancer in first-degree relative(s)					
Absent	369 (79%)	89 (78%)	89 (74%)	100 (85%)	91 (77%)
Present	101 (21%)	25 (22%)	32 (26%)	17 (15%)	27 (23%)
Body mass index					
<25 kg/m^2	209 (48%)	41 (41%)	55 (48%)	58 (53%)	55 (51%)
25 to 29.9 kg/m^2	156 (36%)	35 (35%)	42 (37%)	37 (34%)	42 (39%)
≥30 kg/m^2	68 (16%)	25 (25%)	17 (15%)	15 (14%)	11 (10%)
Tumor location					
Cecum	89 (19%)	27 (24%)	21 (17%)	19 (16%)	22 (19%)
Ascending to transverse colon	139 (30%)	29 (25%)	34 (28%)	42 (36%)	34 (29%)
Descending to sigmoid colon	144 (31%)	29 (25%)	44 (36%)	35 (30%)	36 (31%)
Rectum	97 (21%)	29 (25%)	22 (18%)	20 (17%)	26 (22%)
Tumor differentiation					
Well to moderate	431 (92%)	109 (96%)	106 (88%)	107 (92%)	109 (93%)
Poor	37 (7.9%)	5 (4.4%)	15 (12%)	9 (7.8%)	8 (6.8%)
AJCC disease stage					
I	115 (26%)	29 (27%)	21 (19%)	36 (33%)	29 (26%)
II	158 (36%)	29 (27%)	44 (40%)	38 (35%)	47 (42%)
III	138 (31%)	38 (35%)	37 (34%)	31 (28%)	32 (29%)
IV	29 (6.6%)	13 (12%)	8 (7.3%)	5 (4.6%)	3 (2.7%)
MSI status					
Non-MSI-high	386 (83%)	92 (81%)	96 (81%)	99 (85%)	99 (84%)
MSI-high	81 (17%)	22 (19%)	23 (19%)	17 (15%)	19 (16%)
CIMP status					
Low/negative	384 (82%)	87 (78%)	99 (83%)	101 (86%)	97 (83%)
High	82 (18%)	25 (22%)	21 (18%)	16 (14%)	20 (17%)
Mean LINE-1 methylation level ± SD (%)	61.1 ± 9.6	60.5 ± 10.0	60.4 ± 9.3	60.8 ± 10.1	62.9 ± 9.0
KRAS mutation					
Wild-type	269 (58%)	64 (56%)	65 (55%)	72 (63%)	68 (58%)
Mutant	195 (42%)	50 (44%)	53 (45%)	43 (37%)	49 (42%)
BRAF mutation					
Wild-type	400 (86%)	96 (85%)	105 (88%)	100 (88%)	99 (85%)
Mutant	64 (14%)	17 (15%)	15 (13%)	14 (12%)	18 (15%)
PIK3CA mutation					
Wild-type	353 (82%)	88 (87%)	88 (81%)	91 (83%)	86 (77%)
Mutant	77 (18%)	13 (13%)	20 (19%)	19 (17%)	25 (23%)
Nuclear CTNNB1 (beta-catenin) expression					
Negative	238 (53%)	62 (56%)	60 (51%)	59 (54%)	57 (50%)
Positive	213 (47%)	48 (44%)	58 (49%)	51 (46%)	56 (50%)
PTGS2 (cyclooxygenase-2) expression					
Negative	179 (38%)	48 (42%)	44 (36%)	45 (38%)	42 (36%)
Positive	291 (62%)	66 (58%)	77 (64%)	72 (62%)	76 (64%)
IRS1 expression					
Negative/low	289 (70%)	74 (70%)	83 (76%)	64 (65%)	68 (67%)
High	125 (30%)	31 (30%)	26 (24%)	35 (35%)	33 (33%)
CD3$^+$ cell density					
Quartile 1 (lowest)	111 (25%)	32 (29%)	28 (25%)	29 (26%)	22 (20%)
Quartile 2	111 (25%)	32 (29%)	18 (16%)	32 (28%)	29 (27%)
Quartile 3	112 (25%)	17 (15%)	37 (33%)	26 (23%)	32 (29%)
Quartile 4 (highest)	111 (25%)	29 (26%)	30 (27%)	26 (23%)	26 (24%)
CD8$^+$ cell density					
Quartile 1 (lowest)	110 (25%)	32 (30%)	28 (25%)	31 (28%)	19 (18%)

(continued)

Table 1. (continued)

Characteristic*	All cases (n = 470)	Postdiagnosis physical activity levels			
		Quartile 1 (Lowest) (n = 114)	Quartile 2 (n = 121)	Quartile 3 (n = 117)	Quartile 4 (Highest) (n = 118)
Quartile 2	109 (25%)	31 (29%)	22 (20%)	29 (26%)	27 (25%)
Quartile 3	109 (25%)	20 (19%)	27 (24%)	28 (25%)	34 (32%)
Quartile 4 (highest)	109 (25%)	24 (22%)	34 (31%)	24 (21%)	27 (25%)
$CD45RO^+$ cell density					
Quartile 1 (lowest)	113 (25%)	28 (26%)	27 (23%)	35 (31%)	23 (21%)
Quartile 2	113 (25%)	32 (29%)	33 (28%)	24 (21%)	24 (21%)
Quartile 3	113 (25%)	27 (25%)	29 (25%)	26 (23%)	31 (28%)
Quartile 4 (highest)	112 (25%)	22 (20%)	28 (24%)	28 (25%)	34 (30%)
$FOXP3^+$ cell density					
Quartile 1 (lowest)	107 (25%)	31 (30%)	27 (24%)	24 (24%)	25 (24%)
Quartile 2	106 (25%)	27 (26%)	39 (34%)	18 (18%)	22 (21%)
Quartile 3	106 (25%)	22 (21%)	24 (21%)	25 (25%)	35 (33%)
Quartile 4 (highest)	107 (25%)	24 (23%)	24 (21%)	35 (34%)	24 (23%)

*Percentage (%) indicates the proportion of cases with a specific clinical, pathological, or molecular characteristic of colorectal cancer cases in all cases or in strata of quartiles of postdiagnosis physical activity levels. AJCC = American Joint Committee on Cancer; CIMP = CpG island methylator phenotype; HPFS = Health Professionals Follow-up Study; LINE-1 = long interspersed nucleotide element-1; METS = metabolic equivalent task score; MSI = microsatellite instability; NHS = Nurses' Health Study.

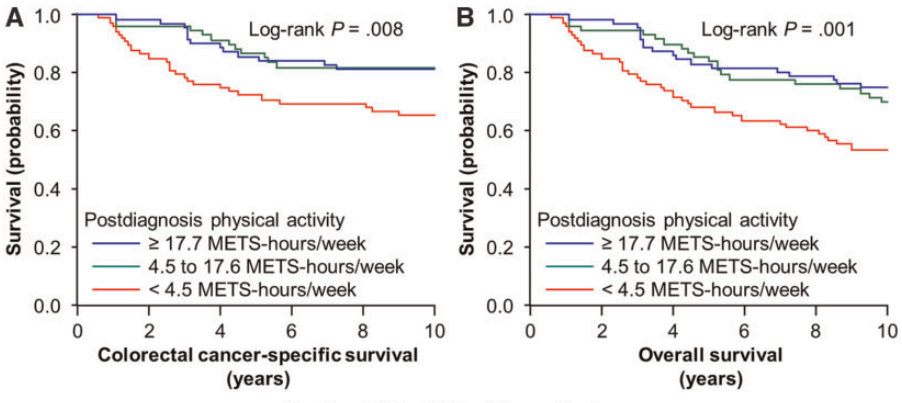
Table 2. Colorectal cancer mortality according to postdiagnosis physical activity levels in all cases or in strata of quartiles of T cell densities

Characteristic	No. of cases	Colorectal cancer-specific mortality HR for a quartile-unit increase of postdiagnosis physical activity levels			Overall mortality HR for a quartile-unit increase of postdiagnosis physical activity levels		
		No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†	No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†
All colorectal cancer cases	470	100	0.77 (0.64 to 0.92)	0.78 (0.64 to 0.95)	275	0.77 (0.69 to 0.86)	0.83 (0.75 to 0.93)
$CD3^+$ cell density							
Quartile 1 (lowest)	111	30	0.61 (0.42 to 0.88)	0.56 (0.38 to 0.83)	69	0.75 (0.59 to 0.95)	0.76 (0.62 to 0.93)
Quartile 2	111	23	0.78 (0.53 to 1.15)	0.80 (0.54 to 1.18)	64	0.68 (0.55 to 0.84)	0.72 (0.58 to 0.89)
Quartile 3	112	25	0.75 (0.49 to 1.14)	0.73 (0.47 to 1.11)	68	0.80 (0.63 to 1.02)	0.83 (0.65 to 1.06)
Quartile 4 (highest)	111	15	1.04 (0.73 to 1.49)	1.14 (0.79 to 1.65)	60	0.85 (0.67 to 1.08)	0.96 (0.76 to 1.21)
$P_{interaction}$‡			.004	<.001		.35	.17
$CD8^+$ cell density							
Quartile 1 (lowest)	110	34	0.67 (0.48 to 0.92)	0.66 (0.47 to 0.94)	66	0.72 (0.56 to 0.92)	0.78 (0.59 to 1.02)
Quartile 2	109	23	0.84 (0.57 to 1.23)	0.81 (0.56 to 1.18)	65	0.81 (0.65 to 1.00)	0.80 (0.65 to 0.99)
Quartile 3	109	18	0.58 (0.37 to 0.90)	0.56 (0.33 to 0.95)	59	0.62 (0.49 to 0.79)	0.75 (0.59 to 0.95)
Quartile 4 (highest)	109	19	1.03 (0.71 to 1.51)	1.02 (0.67 to 1.56)	64	0.87 (0.69 to 1.10)	0.86 (0.70 to 1.05)
$P_{interaction}$‡			.060	.14		.22	.45
$CD45RO^+$ cell density							
Quartile 1 (lowest)	113	33	0.95 (0.69 to 1.31)	0.86 (0.62 to 1.19)	73	0.73 (0.57 to 0.93)	0.77 (0.62 to 0.95)
Quartile 2	113	34	0.70 (0.49 to 1.01)	0.64 (0.44 to 0.95)	69	0.77 (0.60 to 0.97)	0.79 (0.62 to 0.99)
Quartile 3	113	19	0.61 (0.41 to 0.91)	0.65 (0.42 to 1.02)	63	0.74 (0.59 to 0.91)	0.80 (0.65 to 1.00)
Quartile 4 (highest)	112	10	0.87 (0.46 to 1.63)	0.88 (0.44 to 1.72)	60	0.82 (0.66 to 1.03)	0.91 (0.74 to 1.13)
$P_{interaction}$‡			.81	.84		.76	.80
$FOXP3^+$ cell density							
Quartile 1 (lowest)	107	32	0.71 (0.50 to 1.00)	0.75 (0.51 to 1.10)	76	0.67 (0.53 to 0.84)	0.74 (0.58 to 0.94)
Quartile 2	106	23	0.69 (0.48 to 0.99)	0.72 (0.48 to 1.08)	67	0.71 (0.58 to 0.87)	0.75 (0.61 to 0.92)
Quartile 3	106	20	0.94 (0.62 to 1.44)	1.02 (0.68 to 1.51)	56	0.98 (0.75 to 1.27)	1.06 (0.85 to 1.33)
Quartile 4 (highest)	107	13	0.66 (0.42 to 1.03)	0.64 (0.40 to 1.03)	49	0.73 (0.56 to 0.94)	0.77 (0.61 to 0.98)
$P_{interaction}$‡			.46	.33		.73	.31

*IPW was applied to reduce a bias due to the availability of questionnaire data after cancer diagnosis (see Statistical Analysis subsection for details). CI = confidence interval; HR = hazard ratio; IPW = inverse probability weighting.

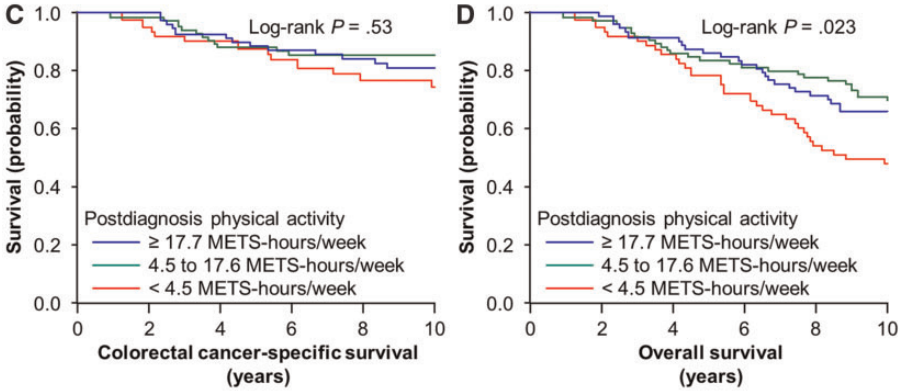
†The multivariable sex-stratified IPW-adjusted Cox regression model initially included age, year of diagnosis, family history of colorectal cancer, body mass index, prediagnosis physical activity, tumor location, tumor differentiation, disease stage, microsatellite instability, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation level, *KRAS* mutation, *BRAF* mutation, *PIK3CA* mutation, nuclear CTNNB1 (beta-catenin) expression, PTGS2 (cyclooxygenase-2) expression, and *IRS1* expression. A backward elimination with a threshold of *P* equal to .05 was used to select variables for the final models. The variables that remained in the final models for analyses stratified by $CD3^+$ cell density are described in Appendix Table A2.

‡$P_{interaction}$ was calculated using the Wald test for the cross-product of postdiagnosis physical activity levels (the median value of each decile category) and each T cell subset (the median value of each decile category) in the sex-stratified IPW-adjusted Cox regression model.



Number at risk: *CD3*⁺ cell-low patients

METS-hours/week	Years					
	0	2	4	6	8	10
≥ 17.7	72	71	63	60	58	55
4.5 to 17.6	66	63	60	52	51	47
< 4.5	84	73	62	56	53	47



Number at risk: *CD3*⁺ cell-high patients

METS-hours/week	Years					
	0	2	4	6	8	10
≥ 17.7	75	74	69	62	54	50
4.5 to 17.6	86	84	75	71	68	61
< 4.5	62	60	54	46	35	31

Figure 1. Inverse probability weighting-adjusted Kaplan-Meier curves of colorectal cancer-specific survival and overall survival according to tertiles of postdiagnosis physical activity levels (<4.5 vs 4.5 to 17.6 vs ≥17.7 METS-h/wk) in strata of $CD3^+$ cell density. The *P* values were calculated using the weighted log-rank test (two-sided). **A and B**), $CD3^+$ cell-low patients; **C and D**), $CD3^+$ cell-high patients. METS = metabolic equivalent task score.

Our previous studies have suggested that the association of postdiagnosis physical activity levels with colorectal cancer survival might differ by nuclear CTNNB1 (42), PTGS2 (39), or *IRS1* expression status (40). Therefore, we performed secondary analyses stratified jointly by $CD3^+$ cell density with nuclear CTNNB1, PTGS2, or *IRS1* expression status (Table 5). Although statistical power was limited, there appeared to be a differential prognostic association by $CD3^+$ cell density across nuclear CTNNB1 expression status and in PTGS2-positive or *IRS1*-negative cases.

In our exploratory analysis, there were no statistically significant interactions between postdiagnosis physical activity levels and any of the lymphocytic reaction patterns examined (Supplementary Table 5, available online).

Discussion

Using two large prospective cohort studies, we tested the hypothesis that the association of postdiagnosis physical activity levels with colorectal cancer survival might differ by levels of tumor-infiltrating T cell subsets ($CD3^+$ cells, $CD8^+$ cells, $CD45RO^+$ cells, or $FOXP3^+$ cells). We found a stronger prognostic association of postdiagnosis physical activity levels for colorectal cancer accompanied by lower levels of $CD3^+$ pan-T cells than for cancer accompanied by higher levels of $CD3^+$ cells. Our results support an interactive effect of physical activity and immune status in the regulation of colorectal cancer progression. A future study is warranted to examine how the association between physical activity levels and colorectal cancer incidence may differ by tumor-infiltrating T cells.

Table 3. Colorectal cancer mortality according to postdiagnosis physical activity levels in all cases or in strata of quartiles of $CD3^+$ cell density by sex

Characteristic	No. of cases	Colorectal cancer-specific mortality HR for a quartile-unit increase of postdiagnosis physical activity levels			Overall mortality HR for a quartile-unit increase of postdiagnosis physical activity levels		
		No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†	No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†
Female							
All colorectal cancer cases	252	55	0.72 (0.55 to 0.93)	0.72 (0.55 to 0.94)	134	0.68 (0.58 to 0.80)	0.68 (0.57 to 0.80)
$CD3^+$ cell density							
Quartile 1 (lowest)	56	15	0.49 (0.27 to 0.87)	0.45 (0.25 to 0.82)	27	0.52 (0.34 to 0.80)	0.57 (0.35 to 0.91)
Quartile 2	61	14	0.78 (0.47 to 1.29)	0.86 (0.50 to 1.48)	30	0.56 (0.39 to 0.79)	0.55 (0.39 to 0.77)
Quartile 3	65	15	0.90 (0.55 to 1.48)	0.74 (0.44 to 1.25)	40	0.92 (0.69 to 1.22)	0.86 (0.65 to 1.15)
Quartile 4 (highest)	53	6	0.55 (0.38 to 0.80)	0.66 (0.44 to 1.00)	29	0.67 (0.50 to 0.91)	0.74 (0.53 to 1.02)
$P_{interaction}$‡			.071	.11		.60	.37
Male							
All colorectal cancer cases	218	45	0.83 (0.65 to 1.07)	0.69 (0.50 to 0.96)	141	0.87 (0.75 to 1.01)	0.98 (0.85 to 1.13)
$CD3^+$ cell density							
Quartile 1 (lowest)	55	15	0.73 (0.46 to 1.15)	0.64 (0.39 to 1.05)	42	0.90 (0.70 to 1.15)	0.93 (0.74 to 1.17)
Quartile 2	50	9	0.77 (0.43 to 1.39)	0.60 (0.32 to 1.13)	34	0.79 (0.62 to 1.01)	0.90 (0.70 to 1.15)
Quartile 3	47	10	0.50 (0.23 to 1.09)	0.55 (0.26 to 1.20)	28	0.63 (0.41 to 0.99)	0.82 (0.53 to 1.26)
Quartile 4 (highest)	58	9	1.58 (0.98 to 2.55)	1.55 (0.86 to 2.80)	31	1.08 (0.76 to 1.54)	1.18 (0.84 to 1.65)
$P_{interaction}$‡			.001	<.001		.25	.27

*IPW was applied to reduce a bias due to the availability of questionnaire data after cancer diagnosis (see "Statistical Analysis" subsection for details). CI = confidence interval; HR = hazard ratio; IPW = inverse probability weighting.

†The multivariable IPW-adjusted Cox regression model initially included age, year of diagnosis, family history of colorectal cancer, body mass index, prediagnosis physical activity, tumor location, tumor differentiation, disease stage, microsatellite instability, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation level, *KRAS* mutation, *BRAF* mutation, *PIK3CA* mutation, nuclear CTNNB1 (beta-catenin) expression, PTGS2 (cyclooxygenase-2) expression, and *IRS1* expression. A backward elimination with a threshold of *P* equal to .05 was used to select variables for the final models.

‡$P_{interaction}$ was calculated using the Wald test for the cross-product of postdiagnosis physical activity levels (the median value of each decile category) and each T cell subset (the median value of each decile category) in the IPW-adjusted Cox regression model.

Table 4. Colorectal cancer mortality according to tertiles (<4.5, 4.5 to 17.6, and ≥17.7 METS-h/wk) of postdiagnosis physical activity levels in strata of $CD3^+$ cell density

Characteristic	No. of cases	Colorectal cancer-specific mortality HR			Overall mortality HR		
		No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†	No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†
$CD3^+$ cell-low‡							
Postdiagnosis physical activity							
<4.5 METS-h/wk	84	29	1 (referent)	1 (referent)	59	1 (referent)	1 (referent)
4.5 to 17.6 METS-h/wk	66	11	0.42 (0.21 to 0.84)	0.37 (0.18 to 0.77)	34	0.53 (0.34 to 0.81)	0.54 (0.35 to 0.84)
≥17.7 METS-h/wk	72	13	0.44 (0.23 to 0.84)	0.33 (0.16 to 0.67)	40	0.53 (0.35 to 0.79)	0.53 (0.35 to 0.79)
$CD3^+$ cell-high‡							
Postdiagnosis physical activity							
<4.5 METS-h/wk	62	13	1 (referent)	1 (referent)	43	1 (referent)	1 (referent)
4.5 to 17.6 METS-h/wk	86	13	0.62 (0.29 to 1.34)	0.58 (0.27 to 1.24)	43	0.58 (0.38 to 0.88)	0.53 (0.35 to 0.81)
≥17.7 METS-h/wk	75	14	0.75 (0.36 to 1.57)	0.62 (0.30 to 1.29)	42	0.63 (0.41 to 0.96)	0.72 (0.47 to 1.11)

*IPW was applied to reduce a bias due to the availability of questionnaire data after cancer diagnosis (see "Statistical Analysis" subsection for details). CI = confidence interval; HR = hazard ratio; IPW = inverse probability weighting; METS = metabolic equivalent task score.

†The multivariable sex-stratified IPW-adjusted Cox regression model initially included age, year of diagnosis, family history of colorectal cancer, body mass index, prediagnosis physical activity, tumor location, tumor differentiation, disease stage, microsatellite instability, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation level, *KRAS* mutation, *BRAF* mutation, *PIK3CA* mutation, nuclear CTNNB1 (beta-catenin) expression, PTGS2 (cyclooxygenase-2) expression, and *IRS1* expression. A backward elimination with a threshold of *P* equal to .05 was used to select variables for the final models.

‡$CD3^+$ cell density was categorized as low vs high by the median value.

Cancer immunotherapies such as immune checkpoint inhibitors have shown considerable promise with durable response (8,10). In colorectal carcinomas, clinical benefits from blockade therapies targeting the *CD274* (PD-L1)-*PDCD1* (PD-1) axis have shown to be greater for MSI-high tumors that are characterized by higher levels of mutation load and immunogenic neoantigens (9). However, a subset of MSI-high colorectal cancer responds poorly to immunotherapies. Intensity of the potential

Table 5. Colorectal cancer mortality according to postdiagnosis physical activity levels in strata of combined $CD3^+$ cell density and nuclear CTNNB1 (beta-catenin) expression, PTGS2 (cyclooxygenase-2) expression, or *IRS1* expression status

		Colorectal cancer-specific mortality HR for a quartile-unit increase of postdiagnosis physical activity levels			Overall mortality HR for a quartile-unit increase of postdiagnosis physical activity levels		
Characteristic	No. of cases	No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†	No. of events	Univariate HR (95% CI)*	Multivariable HR (95% CI)*†
Nuclear CTNNB1 (beta-catenin) expression							
Negative							
$CD3^+$ cell-low‡	109	25	0.60 (0.39 to 0.91)	0.57 (0.37 to 0.88)	65	0.58 (0.46 to 0.74)	0.61 (0.49 to 0.76)
$CD3^+$ cell-high‡	117	22	0.82 (0.58 to 1.16)	0.81 (0.57 to 1.14)	67	0.79 (0.63 to 1.00)	0.89 (0.72 to 1.10)
Positive							
$CD3^+$ cell-low‡	106	28	0.76 (0.55 to 1.06)	0.74 (0.52 to 1.05)	66	0.79 (0.64 to 0.98)	0.87 (0.70 to 1.07)
$CD3^+$ cell-high‡	97	16	0.93 (0.55 to 1.58)	1.03 (0.62 to 1.73)	55	0.91 (0.70 to 1.18)	0.96 (0.75 to 1.24)
PTGS2 (cyclooxygenase-2) expression							
Negative							
$CD3^+$ cell-low‡	74	14	0.62 (0.38 to 1.02)	0.60 (0.37 to 0.99)	43	0.71 (0.55 to 0.91)	0.71 (0.54 to 0.94)
$CD3^+$ cell-high‡	90	14	0.58 (0.36 to 0.96)	0.55 (0.33 to 0.90)	51	0.69 (0.53 to 0.89)	0.78 (0.60 to 1.02)
Positive							
$CD3^+$ cell-low‡	148	39	0.71 (0.52 to 0.96)	0.61 (0.44 to 0.86)	90	0.71 (0.58 to 0.86)	0.74 (0.61 to 0.89)
$CD3^+$ cell-high‡	133	26	1.11 (0.78 to 1.58)	1.06 (0.73 to 1.55)	77	0.95 (0.77 to 1.18)	1.01 (0.82 to 1.24)
IRS1 expression							
Negative/low							
$CD3^+$ cell-low‡	136	38	0.62 (0.44 to 0.86)	0.60 (0.42 to 0.86)	89	0.68 (0.56 to 0.82)	0.71 (0.58 to 0.86)
$CD3^+$ cell-high‡	144	19	0.82 (0.53 to 1.26)	0.76 (0.49 to 1.19)	79	0.87 (0.71 to 1.08)	0.91 (0.74 to 1.12)
High							
$CD3^+$ cell-low‡	57	7	1.73 (1.03 to 2.90)	1.57 (0.93 to 2.64)	30	0.98 (0.72 to 1.34)	0.93 (0.72 to 1.20)
$CD3^+$ cell-high‡	62	19	1.01 (0.69 to 1.46)	1.18 (0.82 to 1.69)	40	0.80 (0.61 to 1.07)	0.89 (0.67 to 1.19)

*IPW was applied to reduce a bias due to the availability of questionnaire data after cancer diagnosis (see "Statistical Analysis" subsection for details). CI = confidence interval; HR = hazard ratio; IPW = inverse probability weighting.

†The multivariable sex-stratified IPW-adjusted Cox regression model initially included age, year of diagnosis, family history of colorectal cancer, body mass index, prediagnosis physical activity, tumor location, tumor differentiation, disease stage, microsatellite instability, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation level, *KRAS* mutation, *BRAF* mutation, *PIK3CA* mutation, nuclear CTNNB1 expression (except for CTNNB1-stratified analyses), PTGS2 expression (except for PTGS2-stratified analyses), and *IRS1* expression (except for *IRS1*-stratified analyses). A backward elimination with a threshold of *P* equal to .05 was used to select variables for the final models.

‡$CD3^+$ cell density was categorized as low vs high by the median value.

anti-tumor immune response is also a major determinant of the response to immunotherapies in colorectal cancer, which is affected by multiple endogenous and exogenous factors, including the gut microbiota (53–55). In this setting, there is an increasing need for integrative analyses of lifestyle factors, tumor features, and host immunity (6). A better understanding of the tumor-immune microenvironment would help us to optimize preventive and treatment strategies through immune modulation.

Evidence indicates the role of energy balance in carcinogenesis (56), and every tumor differs from other tumors (57). In the current study, we found a differential prognostic association of postdiagnosis physical activity levels by $CD3^+$ cell density in colorectal carcinoma tissue. None of the more specific subsets of T cells statistically significantly modified the prognostic association of physical activity. This may indicate that, collectively, the density of pan-T cells (but not specific types of T cells) may indeed modify the prognostic effect of physical activity or that the measurements of the other specific markers of T cells may need further refinements. In addition, the density of $CD3^+$ pan-T cells alone may be a potentially useful biomarker to identify the subpopulation that benefits more from postdiagnosis physical activity. To our knowledge, this is the first exploratory analysis on the interaction between postdiagnosis physical activity and immune response for colorectal cancer mortality. Therefore, our findings need to be validated in independent cohorts. Studies indicate that exercise can alter the number and function of circulating immune cells, including $CD3^+$ cells, $CD4^+$ cells, $CD8^+$ cells, macrophages, and natural killer cells in healthy populations (29). Studies suggest that higher physical activity may increase plasma levels of *ADIPOQ* (adiponectin) (58–60). *ADIPOQ* can suppress inflammatory changes in the tumor microenvironment (61,62). Furthermore, higher levels of *IL6* released from skeletal muscle during exercise may increase levels of cytokines (eg, *IL1R1* and *IL10*) and cortisol, both of which exert anti-inflammatory properties (29,63–65). Preclinical studies suggest that exercise may increase circulating lymphocytes, promote the infiltration of natural killer cells to tumors, and increase apoptosis of cancer cells (29–31). In mouse models for colorectal cancer, exercise was associated with higher expression of cytotoxic T cell marker genes in intestinal mucosal tissue (32). Our population-based data support these mechanistic data, providing evidence for the immunomodulatory effects of physical activity in the regulation of colorectal cancer progression in humans. In the present study, there appeared to be no dose-response relationship between postdiagnosis physical activity levels and patient survival in $CD3^+$ cell-low tumors. This may suggest a possibility of a threshold effect of physical activity for its immunomodulatory anti-tumor influence. However, we have been cautious in interpreting an individual HR estimate comparing each category of physical activity (to the referent category) in each stratum of patients according to T cell

infiltrates, considering multiple hypothesis testing in such assessments. Further research is needed to assess potential dose-response effects of physical activity levels on colorectal cancer mortality in specific tumor types.

There are limitations in the current study. First, limited data on cancer treatments are available in our study populations. However, it was unlikely that treatment strategies were determined by levels of T cell densities, because attending physicians did not have access to such information. Second, the differential availability of questionnaire return for physical activity assessment after colorectal cancer diagnosis might have caused a bias. Thus, we used the IPW method in all survival analyses to reduce this potential bias. Third, available data on cancer recurrence were limited, but given the long follow-up duration of censored cases, colorectal cancer-specific mortality was considered as a reasonable surrogate for colorectal cancer-specific outcomes.

Strengths of the present study include the use of a molecular pathological epidemiology (66–69) database derived from two large prospective cohort studies, which included data on lifestyle factors and tumor molecular and immune characteristics. This integrated database allowed us to examine the interaction between postdiagnosis physical activity levels and tumor-infiltrating T cells while adjusting for a variety of potential confounders. In addition, this database enabled us to adjust for long-term physical activity levels before cancer diagnosis. Participants were enrolled at a large number of hospitals in diverse locations across the United States, which might improve the generalizability of our findings.

The current study suggests that the association of higher levels of postdiagnosis physical activity with better prognosis is stronger for colorectal carcinomas with lower $CD3^+$ cell density than for carcinomas with higher $CD3^+$ cell density. The present study provides evidence for a potential interaction between physical activity and anti-tumor immune response in suppressing colorectal cancer progression. Our findings suggest that the measurement of immune response based on densities of $CD3^+$ in tumor tissue at diagnosis may help select patients who can gain the most benefit from exercise.

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Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols (or root symbols) for genes and gene products, including ADIPOQ, BRAF, CACNA1G, CD3, CD4, CD8, CD274, CDKN2A, CRABP1, CTNNB1, FOXP3, IGF2, IL1R1, IL6, IL10, IRS1, KRAS, MLH1, NEUROG1, PDCD1, PIK3CA, PTGS2, PTPRC, RUNX3, and SOCS1, all of which are described at www.genenames.org. The official symbols are italicized to differentiate from nonitalicized colloquial names that are used along with the official symbols. This format enables readers to familiarize themselves with the official symbols for genes and gene products together with common colloquial names.

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