Density of CD3$^+$ and CD8$^+$ Cells in the Microenvironment of Colorectal Cancer according to Prediagnostic Physical Activity

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ABSTRACT

Background: Physical activity is associated not only with a decreased risk of developing colorectal cancer but also with improved survival. One putative mechanism is the infiltration of immune cells in the tumor microenvironment. Experimental findings suggest that physical activity may mobilize immune cells to the tumor. We hypothesized that higher levels of physical activity prior to colorectal cancer diagnosis are associated with higher densities of tumor-infiltrating T-lymphocytes in colorectal cancer patients.

Methods: The study setting was a northern Swedish population-based cohort, including 109,792 participants with prospectively collected health- and lifestyle-related data. For 592 participants who later developed colorectal cancer, archival tumor tissue samples were used to assess the density of CD3$^+$ and CD8$^+$ cytotoxic T cells by IHC. Odds ratios for associations between self-reported, prediagnostic recreational physical activity and immune cell infiltration were estimated by ordinal logistic regression.

Results: Recreational physical activity >3 times per week was associated with a higher density of CD8$^+$ T cells in the tumor front and center compared with participants reporting no recreational physical activity. Odds ratios were 2.77 (95% CI, 1.21–6.35) and 2.85 (95% CI, 1.28–6.33) for the tumor front and center, respectively, after adjustment for sex, age at diagnosis, and tumor stage. The risk estimates were consistent after additional adjustment for several potential confounders. For CD3, no clear associations were found.

Conclusions: Physical activity may promote the infiltration of CD8$^+$ immune cells in the tumor microenvironment of colorectal cancer.

Impact: The study provides some evidence on how physical activity may alter the prognosis in colorectal cancer.

Introduction

Physical activity reduces the risk of colorectal cancer (1–3) and has been associated with improved survival (4–7). Evidence suggests that these beneficial effects on survival apply to both pre- and postdiagnostic physical activity (4, 5).

The prognosis of colorectal cancer is modulated by the infiltration of immune cells in the tumor microenvironment. A high density of leukocytes expressing the general T-cell marker, CD3, and/or the cytotoxic T-cell marker, CD8, in the tumor microenvironment is associated with longer patient survival (8–14). T-cell densities are also lower in the tumor microenvironment of metastatic compared with nonmetastatic colorectal cancer at diagnosis (11), possibly suggesting that tumor-infiltrating T cells have a role in preventing the dissemination of the disease.

The biological mechanisms behind the inverse relationship between physical activity and colorectal cancer have not been fully elucidated (15), but evidence suggests that physical activity may affect the immune system and immune cell infiltration of the tumor microenvironment in a prognostically favorable manner (16, 17). An acute bout of exercise mobilizes lymphocytes to the bloodstream (18) and to nonlymphoid organs (19). Furthermore, aerobic fitness is associated with lower proportions of senescent CD3$^+$ and CD8$^+$ cells in the blood and a higher proportion of naïve CD3$^+$ and CD8$^+$ (20). Associations between physical activity and tumor-infiltrating T cells have recently gained scientific attention. In animal models, physical activity has been associated with slower tumor growth (21), alteration of the CD8$^+$ cells in a cytotoxic and antitumoral efficacy, and increased numbers of CD8$^+$ cells in the tumor microenvironment (17, 22, 23).

The development of a neoplastic polyp into colorectal cancer can take up to 20 years (24), allowing for a long period of exposure to, and potential modulation by, exogenous and endogenous factors. We hypothesized that physical activity during this asymptomatic, prediagnostic phase of tumor progression may lead to higher densities of tumor-infiltrating T cells in colorectal cancer, which in turn improves cancer-specific survival.

In this study of colorectal cancer patients from a population-based cohort, we investigated self-reported, prediagnostic physical activity in relation to the densities of CD3$^+$ and CD8$^+$ immune cells in the tumor microenvironment.

Materials and Methods

Study cohort and study population

We conducted a cohort study using retrospectively collected colorectal cancer patients included in a prospectively sampled, population-based cohort from northern Sweden, the Västerbotten Intervention

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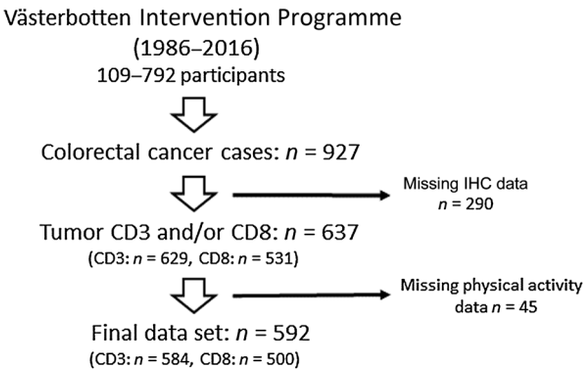

Figure 1.

Selection of participants who developed colorectal cancer after participating in the Västerbotten Intervention Programme cohort and for whom both tumor-infiltrating lymphocyte data and prediagnostic physical activity data were available.

Programme (VIP; ref. 25). In brief, VIP is an ongoing health screening program in Region Västerbotten, initiated in 1986. All inhabitants are invited to participate at ages 40, 50, and 60. The program has a participation rate of approximately 65% and includes a health examination performed by a health professional, a questionnaire on diet, health, and lifestyle, and a blood sample (25). VIP is the largest cohort in the umbrella cohort referred to as the Northern Sweden Health and Disease Study.

The selection of participants for the present study is presented in **Fig. 1**. At the final recruitment date for the present study, January 19, 2016, the VIP included 109,792 participants (**Fig. 1**). The Swedish Cancer Registry was used to identify colorectal cancer cases (International Classification of Disease, ICD-10, codes C18.0 and C18.2-18.9 for colon cancer and C19.9 and C20.9 for rectal cancer). Diagnoses were verified, and data on tumor stage and anatomic tumor site were collected using the Swedish Colorectal Cancer Registry and, when necessary, individual patient records. A total of 927 colorectal cancer cases with prediagnostic participation in VIP were available for inclusion in the present study. Of these, formalin-fixed, paraffin-embedded tumor specimens from 637 patients were available and successfully analyzed for CD3 and/or CD8, including 592 with physical activity data.

Baseline study variables

The exposure variable in this study was self-reported recreational physical activity estimated on a five-level scale ("never," "now, and then," "1–2 times per week," "2–3 times per week," ">3 times per week") at baseline. The variable is based on a single questionnaire item, phrased "How often have you exercised in exercise clothing over the past three months, with the intent to improve your fitness and/or well-being?" The questionnaire for physical activity in VIP has not been specifically validated, but it was included in a multicenter validation of self-reported physical activity including the composite Cambridge index (26).

Other baseline variables used included age at baseline, body mass index (BMI, kg/m^2), diabetes (dichotomous), self-reported smoking status (never smoker, previous smoker, or current smoker), self-reported alcohol intake (none, above the sex-specific median and under the sex-specific median), self-reported education level (no secondary, secondary, or post-secondary education), blood pressure, and plasma C-reactive protein concentrations (CRP, mg/l). Alcohol intake data were obtained from a validated food frequency questionnaire (27) and divided into zero intake or above/below sex-specific medians in grams/day (4.88 g/day for men and 0.95 g/day for women, based on the initial colorectal cancer case population, $n = 927$). Diabetes was defined as self-reported diabetes (yes/no) and/or fasting blood glucose level of >7.0 mmol/L and/or >12.2 mmol/L at two hours after the oral glucose tolerance test. CRP was analyzed quantitatively by immunoassay (Meso-Scale Discovery). When participants had two observations in VIP prior to colorectal cancer diagnosis, the observation closest to the date of diagnosis was selected as the baseline for the exposure variables in this study.

Tumor tissue analysis

Tumor tissue samples collected during routine clinical diagnostics were acquired from the regional health care biobank in Västerbotten (Biobanken Norr). According to routine procedures, diagnostic colorectal cancer specimens obtained after primary tissue resection were fixed in 4% formaldehyde and embedded in paraffin, and long-termed stored at room temperature. From each patient, one 4-µm section was cut, dried, dewaxed, and rehydrated. For IHC procedures, a staining machine (Ventana BenchMark Ultra, Ventana Medical Systems, Inc.) was used with the CC1 standard pretreatment and the iVIEW DAB detection kit (Ventana Medical Systems, Inc.) for visualization. Anti-CD8 polyclonal antibody (clone C8/144B: Dako) and primary polyclonal CD3 antibody (Dako) were used at a dilution of 1:50. The slides were counterstained with hematoxylin. The cases were diagnosed between 1992 and 2016, and the median storage time was approximately 11 years.

The immune cell density was scored as (1) no or sporadic; (2) moderate numbers; (3) abundant occurrence; or (4) highly abundant cells. This scoring was assigned for both cell types in three locations within each tumor: the tumor front (cells localized in stroma adjacent to the invasive margin of the tumor), the tumor center (cells localized in the stroma within the tumor mass), and the intraepithelial compartment (cells localized within tumor cell nests). A total score for each cell type and tumor was calculated as the sum of the scores from each location, ranging from 3 to 12. The total score was classified as low (3–4), intermediate (5–6), or high (7–12), in accordance with previous studies (8, 9, 28). Immune infiltration was scored by one observer (BG) under the supervision of a senior consultant in gastrointestinal pathology (RP). A subsample of 35 consecutively selected tumors was reexamined by a second observer to assess interobserver agreement (weighted Cohen Kappa of 0.61–0.87, with 0.71 for total score).

Statistical analyses

Baseline characteristics were compared using the χ^2 test for ordinal and categorical variables. Continuous variables were defined as normally distributed or not using a Shapiro–Wilks test. All continuous variables were nonnormally distributed, and the Kruskal–Wallis test was therefore used for continuous variables.

Odds ratios (OR) were calculated using ordinal logistic regression and generalized ordinal logistic regression. For each multivariable model, we tested if the proportional odds assumption was met using the Brant test. When violated, generalized ordinal logistic regression was used (29) with relaxing of the proportional odds assumption for variables that violated the assumption. Calculations were made for both cell types ($CD3^+$ and $CD8^+$) in each position (tumor front, center, intraepithelial, and total score). In order to test for a dose–response relationship, P trends were calculated by including the physical activity categories (numbered 1–5 for lowest to highest) as a continuous variable in the regression models.

Three regression models were constructed: crude (univariable), minimally adjusted (sex, age at diagnosis, and tumor stage), and fully adjusted. Physical activity was the exposure variable and treated as an ordinal variable. Several potential confounders of associations between physical activity and tumor immune cell infiltration were available in the data set and considered for inclusion in the fully adjusted model. As summarized in a directed acyclic graph in Supplementary Fig. S1, these included the variables in the minimally adjusted model as well as baseline age, tumor site, BMI, smoking status, diabetes status, alcohol intake, education level, systolic and diastolic blood pressure, CRP, and year of diagnosis. Although additional tumor data were available for some or all of the patients in this study (microsatellite instability status, *BRAF*, and *KRAS* mutations, and CpG island methylator phenotype), we did not consider them to be potential confounders, given the current lack of theory or evidence for an independent association with physical activity (30–33). Variables were categorized as described in the baseline study variables section or left as continuous variables if not otherwise specified. Covariates were selected for the fully adjusted model using bivariable ordinal logistic regressions. Variables that altered the beta-coefficient for the association between recreational physical activity (as a continuous variable) and $CD3^+$ or $CD8^+$ total score by more than 10% were included in the respective fully adjusted model (34). Collinearity was tested using variance inflation factor. Variance inflation factor above 5 was considered high collinearity. No collinearity was observed in the minimally or fully adjusted models. The additional covariates included in the final fully adjusted models were, for $CD8^+$: tumor site, baseline age, CRP, and year of diagnosis; for $CD3^+$: tumor site, BMI, smoking status, systolic blood pressure, and alcohol intake.

Missing data for covariates in the risk analyses were imputed, using sex-specific medians for nonnormally distributed continuous variables, and sex-specific modes for categorical variables based on the final study population ($n = 592$). Due to a higher number of missing observations for alcohol ($n = 29$), missing data were included as a separate category in the regression models.

Secondary analyses included three prespecified subgroup analyses in ordinal logistic regression models. First, stratification by sex was conducted as a sensitivity analysis. Secondly, given our hypothesis of a role for physical activity in tumor progression, we stratified by follow-up time from baseline to diagnosis (at a mean of 9.4 years). Finally, in consideration of the close interrelationship between physical activity and body size, with respect to both colorectal cancer and the immune response and inflammation (35, 36), we ran subgroup analyses stratifying at the BMI cutoff-point between normal weight and overweight (BMI $\geq$ 25 kg/m^2). All subgroup analyses were conducted using the minimally adjusted model.

All statistical analyses were conducted using STATA version 15.1 (StataCorp LP).

This study was approved by the Regional Ethics Review Board in Umeå, Sweden (2015-243-31 and amendment 2020-00081).

Results

In the final study population of 592 colorectal cancer cases (**Table 1**), 584 (98.6%) had CD3 IHC data, and 500 (84.5%) had CD8 IHC data available.

The proportion of women was 49.3%, and the mean age at diagnosis was 65.6 years. The median time from baseline to diagnosis was 9.4 years (25th–75th percentile 5.2–13.3 years).

Higher $CD3^+$ and $CD8^+$ total scores were associated with female gender, right-sided colon cancer, and less-advanced tumor stage (**Table 2**).

In the ordinal logistic regression models, physical activity >3 times per week was associated with a higher density of $CD8^+$ immune cells in the tumor front and center (**Table 3**). ORs were 2.77 (95% CI, 1.21–6.35) and 2.85 (95% CI, 1.28–6.33) for the tumor front and center, respectively, after adjustment for sex, age at diagnosis, and tumor stage. These results were consistent and remained statistically significant after adjustment for additional potential confounders in the fully adjusted model. For the $CD8^+$ total score, the ORs were attenuated to approximately 2 and no longer statistically significant, due to a null association for $CD8^+$ intraepithelial immune cell density. *P* trends were not significant.

For $CD3^+$, there were no clear associations between recreational physical activity and immune cell infiltration, with ORs generally around 1 (**Table 3**).

In analyses stratified by sex, time from baseline to colorectal cancer diagnosis, and BMI (**Table 4**), subgroup results were similar to each other, and the overall findings in **Table 3**.

Discussion

In this population-based study of 592 colorectal cancer cases from a prospectively sampled cohort, the highest category of prediagnostic self-reported recreational physical activity (>3 times/week) was associated with a higher density of $CD8^+$ immune cells in the tumor front and center. Overall, these findings provide some suggestive support for our primary hypothesis.

In our study, higher immune cell infiltration at higher recreational physical activity was observed for $CD8^+$ but not for $CD3^+$ cells. Animal studies have shown that physical activity mobilizes $CD8^+$ cells to the bloodstream (18), as well as to the tumor microenvironment (17, 22, 23). In one recent murine study, physical activity increased the infiltration of $CD8^+$ cells in the tumor microenvironment, and the presence of $CD8^+$ cells was associated with enhanced survival and suppressed tumor growth (23). Similar results have not been demonstrated for $CD3^+$ cells. Because $CD3^+$ is a pan T-cell marker, whereas $CD8^+$ is a marker of the subpopulation of cytotoxic T cells, physical activity appears to influence different T-cell populations in different patterns. Our results indicate that the effect of prediagnostic physical activity on tumor-infiltrating lymphocytes may be more pronounced for $CD8^+$ compared with $CD3^+$ immune cells. A recent study by Koh and colleagues (37) hypothesized that the survival benefits seen with increased postdiagnosis physical activity might be stronger in tumors with lower T-cell densities. Koh and colleagues concluded this association between postdiagnosis physical activity and improved survival was seen in tumors with lower $CD3^+$ T-cell densities, but no association was seen for $CD8^+$. Their results in perspective to the present study suggest that physical activity alters the immune infiltration in the microenvironment of the tumor by increasing the densities of $CD8^+$ cells. However, in patients with low $CD3^+$ density tumors, postdiagnosis physical activity has the most beneficial survival effects.

The lack of association between recreational physical activity and intraepithelial infiltration of $CD8^+$ cells was not consistent with our hypothesis or with the results for $CD8^+$ in the tumor front and center. There are, broadly, four subsets of $CD8^+$ T cells, ranging from naïve to effector memory subtypes which reflect different maturation steps. These cell types express different surface proteins (38, 39). The naïve $CD8^+$ T cells have not been introduced to an antigen, whereas the

Table 1. Baseline and clinical variables, according to levels of self-reported prediagnostic physical activity, in the final data set of 592 colorectal cancer cases and for an additional 45 cases excluded due to missing physical activity data.

		Recreational physical activity						
	Total ***n*** **= 637[a]**	**Never** ***n*** **= 294**	**Now and then** ***n*** **= 147**	**1–2 times/wk** ***n*** **= 71**	**2–3 times/wk** ***n*** **= 51**	**>3 times/wk** ***n*** **= 29**	**Missing[b]** ***n*** **= 45**	***P*[b]**
Sex, *n* (%)								
Man	323	153 (52.0)	82 (55.8)	33 (46.5)	24 (47.1)	13 (44.8)	18 (40.0)	0.601[c]
Woman	314	141 (48.0)	65 (44.2)	38 (53.5)	27 (52.9)	16 (55.2)	27 (60.0)	
Age at baseline[d]	637	59.9 (50.2–60.1)	59.9 (50.1–60.1)	59.8 (50.0–60.0)	59.9 (50.0–60.1)	59.9 (50.4–60.0)	59.5 (50.0–60.0)	0.335[e]
Age at diagnosis, *n* (%)								
<55	86	31 (10.5)	23 (15.7)	15 (21.1)	8 (15.7)	3 (10.3)	6 (13.3)	0.326[c]
55–65	182	85 (28.9)	39 (26.5)	23 (32.4)	14 (27.5)	13 (44.8)	8 (17.8)	
65–75	284	135 (45.9)	65 (44.2)	25 (35.2)	25 (49.0)	11 (37.9)	23 (51.1)	
>75	85	43 (14.6)	20 (13.6)	8 (11.3)	4 (7.8)	2 (6.9)	8 (17.8)	
Time from baseline to diagnosis, years[d]	637	9.44 (5.38–13.7)	8.92 (5.75–13.2)	8.30 (4.63–13.3)	6.49 (4.61–12.0)	6.15 (3.50–8.65)	12.4 (8.44–18.8)	0.009[e]
BMI[f], kg/m^2	634	27.0 (3.9)	26.5 (3.7)	26.3 (4.4)	26.7 (4.9)	26.5 (5.3)	26.0 (4.3)	0.215[e]
Missing, (*n*)	3	2	0	0	0	0	0	
Diabetes, *n* (%)								
No	561	263 (89.8)	126 (86.3)	63 (88.7)	46 (92.0)	26 (89.7)	37 (82.2)	0.785[c]
Yes	73	30 (10.2)	20 (13.7)	8 (11.3)	4 (8.0)	3 (10.3)	8 (17.8)	
Missing[b], (*n*)	3	1	1	0	1	0	0	
Smoker, *n* (%)								
Never smoker	258	106 (36.8)	72 (50.4)	28 (40.0)	21 (42.0)	15 (57.7)	16 (38.1)	0.152[c]
Ex-smoker	246	128 (44.4)	47 (32.9)	33 (47.1)	20 (40.0)	8 (30.8)	10 (23.8)	
Current smoker	115	54 (18.8)	24 (16.8)	9 (12.9)	9 (18.0)	3 (11.5)	16 (38.1)	
Missing[b], (*n*)	18	6	4	1	1	3	3	
Alcohol intake, *n* (%)								
None	48	25 (8.9)	9 (6.5)	2 (2.9)	6 (12.2)	4 (14.8)	2 (33.3)	0.080[c]
Under median[f]	255	131 (46.8)	70 (50.4)	26 (38.2)	18 (36.7)	7 (25.9)	3 (50.0)	
Above median[f]	266	124 (44.3)	60 (43.2)	40 (58.8)	25 (51.0)	16 (59.3)	1 (16.7)	
Missing[b], (*n*)	68	14	8	3	2	2	39	
Education level, *n* (%)								
No secondary	433	223 (76.6)	99 (67.8)	38 (54.3)	32 (64.0)	14 (50.0)	27 (73.0)	0.002[c]
Secondary	90	31 (10.7)	26 (17.8)	16 (22.9)	7 (14.0)	5 (17.9)	5 (13.5)	
Post-secondary	99	37 (12.7)	21 (14.4)	16 (22.9)	11 (22.0)	9 (32.1)	5 (13.5)	
Missing[b], (*n*)	15	3	1	1	1	1	8	
Systolic BP[d], mm Hg	627	135 (122–148)	130 (120–142)	131 (118–140)	125 (116–140)	130 (120–145)	135 (120–145)	0.043[e]
Missing[b], (*n*)	10	7	1	0	1	0	1	
Diastolic BP[d], mm Hg	625	85 (76–90)	84 (76–90)	80 (75–90)	80 (70–88)	81 (74–90)	84 (74–90)	0.287[e]
Missing[b], (*n*)	12	8	1	0	1	1	1	
CRP[d], mg/L	637	1.87 (0.77–3.67)	1.59 (0.81–2.88)	1.13 (0.64–2.73)	1.86 (0.70–4.59)	1.27 (0.71–2.97)	1.39 (0.62–2.71)	0.231[e]
Tumor site, *n* (%)								
Right colon	225	103 (35.2)	56 (38.1)	22 (31.0)	13 (25.5)	8 (27.6)	23 (51.1)	0.234[c]
Left colon	194	97 (33.1)	47 (32.0)	23 (32.4)	12 (23.5)	8 (27.6)	7 (15.6)	
Rectum	217	93 (31.7)	44 (29.9)	26 (36.6)	26 (51.0)	13 (44.8)	15 (33.3)	
Missing[b], (*n*)	1	1	0	0	0	0	0	
Stage, *n* (%)								
I and II	345	160 (55.9)	80 (55.2)	40 (56.3)	29 (58.0)	14 (48.3)	22 (50.0)	0.940[c]
III and IV	280	126 (44.1)	65 (44.8)	31 (43.7)	21 (42.0)	15 (51.7)	22 (50.0)	
Missing[b], (*n*)	12	8	2	0	1	0	1	

Abbreviations: BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; wk, week.
[a]Final data set (*n* = 592) and cases excluded due to missing recreational physical activity data (*n* = 45).
[b]Missing categories not included in statistical comparisons.
[c]Chi-square test.
[d]Results displayed as median (25–75 percentile).
[e]Kruskal–Wallis test.
[f]Sex-specific medians, 4.88 g/day for men and 0.95 g/day for women, calculated from the initial colorectal cancer population diagnosed after participation In the cohort (*n* = 927).

effector memory subtypes are those that, to the highest extent, exert the cytotoxic function. A previous study on 13 healthy and active humans showed that effector memory subtypes were mobilized to a greater extent than the naïve cells following exercise (18). Different subtypes also express different adhesion molecules and thus infiltrate different tissues to a different extent. Perhaps, in a similar manner, different $CD8^+$ subtypes respond differently to physical activity and infiltrate different localizations in the tumor microenvironment in colorectal cancer.

Table 2. Clinical variables and characteristics of colorectal cancer patients according to $CD3^+$ and $CD8^+$ total score.

	$CD8^+$ total score[a]					$CD3^+$ total score[a]				
	***N* = 519**	**1 (low) *n* = 207**	**2 (med) *n* = 139**	**3 (high) *n* = 173**	***P* value**	***N* = 617**	**1 (low) *n* = 191**	**2 (med) *n* = 174**	**3 (high) *n* = 252**	***P* value**
Age at diagnosis, *n* (%)										
<55	58	25 (12.1)	20 (14.4)	13 (7.5)	0.100[b]	83	26 (13.6)	29 (16.7)	28 (11.1)	0.057[b]
55–65	132	57 (27.5)	34 (24.5)	41 (23.7)		176	61 (31.9)	51 (29.3)	64 (25.4)	
65–75	244	101 (48.8)	62 (44.6)	81 (46.8)		274	86 (45.0)	75 (43.1)	113 (44.8)	
>75	85	24 (11.6)	23 (16.6)	38 (22.0)		84	18 (9.4)	19 (10.9)	47 (18.7)	
Sex, *n* (%)										
Man	254	119 (57.5)	66 (47.5)	69 (39.9)	0.003[b]	313	98 (51.3)	107 (61.5)	108 (42.9)	0.001[b]
Woman	265	88 (42.5)	73 (52.5)	104 (60.1)		304	93 (48.7)	67 (38.5)	144 (57.1)	
Tumor site, *n* (%)										
Right colon	195	68 (32.9)	42 (30.4)	85 (49.1)	0.001[b]	224	66 (34.6)	56 (32.2)	102 (40.6)	0.051[b]
Left colon	161	64 (30.9)	46 (33.3)	51 (29.5)		190	55 (28.8)	51 (29.3)	84 (33.5)	
Rectum	162	75 (36.2)	50 (36.2)	37 (21.4)		202	70 (36.7)	67 (38.5)	65 (25.9)	
Missing[c]	1	0	1	0		1	0	0	1	
Stage, *n* (%)										
I and II	284	95 (46.6)	75 (54.7)	114 (68.3)	<0.001[b]	334	74 (39.0)	94 (54.3)	166 (68.3)	<0.001[b]
III and IV	224	109 (53.4)	62 (45.3)	53 (31.7)		272	116 (61.1)	79 (45.7)	77 (31.7)	
Missing[c]	11	3	2	6		11	1	1	9	

Abbreviations: CD, cluster of differentiation; med, medium.
[a]Calculated as the sum of the scores from each location, ranging from 3 to 12. The total score was classified as low (3–4), intermediate (5–6), or high (7–12).
[b]Chi-square test.
[c]Missing categories not included in statistical comparisons.

In our study, the association between higher recreational physical activity and higher tumor immune cell infiltration was primarily seen in participants reporting recreational physical activity >3 times per week (**Table 3**), and *P* trends were not statistically significant. Physical activity may, therefore, have a threshold rather than a dose–response relationship with tumor immune cell infiltration, occurring at higher physical activity levels. The amount of physical activity required to lower the risk and mortality of colorectal cancer is not known, and the evidence to date does not permit distinction between a threshold or dose–response effect (1, 5, 40–42).

Physical activity is associated with a lower risk of, and improved survival in, colorectal cancer (3, 4). Colorectal cancer is also associated with several lifestyle factors such as obesity, diabetes, and intake of processed meat (43). The development of a neoplastic polyp into a colorectal adenocarcinoma can take up to 20 years (24). It is plausible that differences in the tumor microenvironment begin long before the colorectal cancer is diagnosed. Our baseline data consist of self-reported physical activity data prior to the date of diagnosis. We do not know if the participants maintain their physical activity level until tumor tissue sample collection, but in cases with shorter time from baseline to diagnosis, the reported physical activity is more likely to have occurred in the presence of a neoplastic lesion. Our subgroup analysis of time from baseline to diagnosis showed no material differences compared with the total data set, except the loss of significant results in some analyses (**Table 4**), possibly due to loss of statistical power.

Obesity affects the immune system and is associated with chronic, low-grade, systemic inflammation (35). There is also an established association between overweight and increased risk of colorectal cancer (44). A previous study investigated the association between BMI and immune infiltration of $CD3^+$, $CD8^+$, $CD45RO^+$, and $FOXP3^+$ cells in the tumor microenvironment in colorectal cancer and showed no association (45). In contrast, another study reported an association between obesity and higher immune infiltration of $CD8^+$ cells in the colorectal cancer tumor microenvironment (46). Therefore, in addition to considering BMI as a potential confounder, we conducted a subgroup analysis stratifying at a cutoff of BMI 25 kg/m^2. The results were consistent with those for the total data set (**Table 4**), suggesting that the immune infiltration of $CD8^+$ is associated with physical activity regardless of BMI.

There are limitations in the present study. Probably the most important weakness was the use of a single self-reported recreational physical activity questionnaire item as the primary exposure variable. Objective measurements of usual physical activity, such as with an accelerometer, or a fitness test (e.g., 6-minute walking test or VO2-max cycling test) as a proxy of usual physical activity levels, are more reliable than self-reported estimations (47). Self-reported physical activity may overestimate the actual physical activity when compared with accelerometer data (48, 49). The VIP questionnaire includes several questions on physical activity, including occupational physical activity, as well as several specific types of activities. However, they are difficult to combine (into MET-hours/week, for example) and have varied over the years. Also, occupational physical activity may be more reflective of other factors than overall physical activity, e.g., socioeconomic status (with accompanying differences in other lifestyle-related risk factors), as well as gender differences in type of occupational physical activity, and results have been conflicting regarding whether occupation affects how physically active one is (50, 51). In order to address our hypothesis while taking into consideration the risk of chance findings due to multiple testing if several exposure variables were to be used, we selected recreational activity as the variable most likely to best represent overall physical activity level and to best capture moderate to vigorous activity. The questionnaire for physical activity in the VIP has not been specifically validated, but it was included in a multicenter validation of self-reported physical activity including the composite Cambridge index (26). Another weakness, common in molecular epidemiology studies such as this one, is the risk of selection bias due to missing tumor data. Although essentially no patients were unaccounted for in our study, IHC data were lacking for approximately one third of the potentially eligible cases, due largely to lack of tumor

Table 3. Odds ratios (95% confidence intervals) for self-reported, prediagnostic, recreational physical activity in relation to tumor immune cell infiltration in colorectal cancer[a].

		Recreational physical activity					*P*
		Never	Now and then	1–2 times/wk	2–3 times/wk	>3 times/wk	trend[b]
CD8[a]							
Front[c]	Cases (*n*)	237	125	55	49	22	
	Crude	1 (Ref)	1.31 (0.88–1.94)	1.00 (0.58–1.70)	0.74 (0.42–1.30)	2.33 (1.05–5.18)	0.593
	Minimally adj.[d]	1 (Ref)	1.36 (0.92–2.02)	1.02 (0.59–1.78)	0.75 (0.42–1.32)	2.77 (1.21–6.35)	0.449
	Fully adj.[e]	1 (Ref)	1.32 (0.89–1.98)	1.13 (0.64–1.97)	0.81 (0.45–1.45)	2.91 (1.25–6.75)	0.264
Center[f]	Cases (*n*)	247	127	55	49	22	
	Crude	1 (Ref)	1.25 (0.84–1.86)	0.98 (0.56–1.72)	0.98 (0.55–1.73)	2.25 (1.03–4.92)	0.251
	Minimally adj.[d]	1 (Ref)	1.38 (0.92–2.06)	1.03 (0.58–1.81)	1.05 (0.59–1.87)	2.85 (1.28–6.33)	0.107
	Fully adj.[e]	1 (Ref)	1.39 (0.93–2.08)	1.05 (0.59–1.86)	1.04 (0.58–1.86)	2.92 (1.31–6.50)	0.095
Intra-ep.[g]	Cases (*n*)	247	127	55	49	22	
	Crude	1 (Ref)	1.03 (0.67–1.58)	1.17 (0.67–2.03)	1.22 (0.67–2.21)	0.95 (0.41–2.20)	0.624
	Minimally adj.[d,h]						
	1 vs. 2, 3, 4	1 (Ref)	0.97 (0.62–1.54)	1.20 (0.65–2.13)	1.17 (0.61–2.23)	1.18 (0.47–2.97)	0.497
	1, 2 vs. 3, 4	1 (Ref)	1.66 (1.00–2.78)	1.13 (0.55–2.35)	2.09 (1.04–4.19)	1.42 (0.48–4.16)	0.102
	1, 2, 3 vs. 4	1 (Ref)	1.38 (0.75–2.56)	1.25 (0.53–2.92)	1.14 (0.46–2.81)	0.37 (0.05–2.88)	0.844
	Fully adj.[e,i]						
	1, 2, 3, and 4	1 (Ref)	0.95 (0.60–1.50)	1.25 (0.67–2.31)	1.16 (0.60–2.25)	1.18 (0.46–3.00)	0.485
	1, 2 vs. 3, 4	1 (Ref)	1.55 (0.92–2.59)	1.21 (0.58–2.51)	2.02 (1.00–4.10)	1.49 (0.51–4.36)	0.094
	1, 2, 3 vs. 4	1 (Ref)	1.28 (0.69–2.39)	1.25 (0.52–2.99)	1.14 (0.45–2.87)	0.44 (0.06–3.44)	0.946
TS[j]	Cases (*n*)	237	125	55	49	22	
	Crude	1 (Ref)	1.09 (0.73–1.63)	1.06 (0.62–1.81)	0.97 (0.54–1.75)	1.82 (0.81–4.09)	0.386
	Minimally adj.[d]	1 (Ref)	1.12 (0.75–1.69)	1.10 (0.63–1.91)	1.00 (0.55–1.82)	2.19 (0.55–5.10)	0.247
	Fully adj.[e]	1 (Ref)	1.11 (0.74–1.68)	1.16 (0.66–2.02)	1.03 (0.55–1.90)	2.34 (0.99–5.52)	0.185
CD3[a]							
Front[c]	Cases (*n*)	282	144	68	51	29	
	Crude	1 (Ref)	1.16 (0.80–1.67)	0.95 (0.59–1.53)	1.08 (0.64–1.82)	0.76 (0.38–1.52)	0.722
	Minimally adj.[d,k]						
	1, 2, 3, and 4	1 (Ref)	1.02 (0.64–1.63)	0.95 (0.51–1.75)	1.31 (0.63–2.71)	0.77 (0.33–1.80)	0.992
	1, 2 vs. 3, 4	1 (Ref)	1.09 (0.71–1.66)	0.85 (0.48–1.50)	1.11 (0.59–2.08)	0.69 (0.29–1.67)	0.635
	1, 2, 3 vs. 4	1 (Ref)	2.27 (1.29–4.01)	1.18 (0.51–2.74)	0.73 (0.24–2.20)	1.73 (0.55–5.46)	0.643
	Fully adj.[k,l]						
	1 vs. 2, 3, 4	1 (Ref)	0.91 (0.57–1.47)	0.97 (0.52–1.80)	1.54 (0.74–3.24)	0.87 (0.36–2.10)	0.686
	1, 2 vs. 3, 4	1 (Ref)	0.97 (0.63–1.50)	0.89 (0.50–1.58)	1.30 (0.68–2.47)	0.75 (0.30–1.85)	0.949
	1, 2, 3 vs. 4	1 (Ref)	2.12 (1.19–3.77)	1.25 (0.54–2.92)	0.81 (0.27–2.47)	1.86 (0.57–6.03)	0.488
Center[f]	Cases (*n*)	290	145	69	51	29	
	Crude	1 (Ref)	1.08 (0.75–1.54)	1.02 (0.63–1.65)	1.44 (0.85–2.42)	1.43 (0.73–2.83)	0.153
	Minimally adj.[d,m]						
	1 vs. 2, 3, 4	1 (Ref)	1.37 (0.83–2.28)	0.92 (0.50–1.70)	1.88 (0.80–4.39)	2.20 (0.73–6.64)	0.965
	1, 2 vs. 3, 4	1 (Ref)	0.88 (0.58–1.34)	1.04 (0.60–1.80)	1.43 (0.78–2.63)	1.60 (0.72–3.54)	0.172
	1, 2, 3 vs. 4	1 (Ref)	1.53 (0.88–2.66)	1.19 (0.57–2.50)	1.04 (0.43–2.49)	1.74 (0.61–4.98)	0.392
	Fully adj.[l]	1 (Ref)	1.07 (0.74–1.55)	0.98 (0.60–1.60)	1.41 (0.83–2.42)	1.66 (0.81–3.41)	0.132
Intra-ep.[g]	Cases (*n*)	290	145	69	51	28	
	Crude	1 (Ref)	0.99 (0.67–1.45)	0.97 (0.59–1.59)	1.35 (0.77–2.36)	0.73 (0.33–1.61)	0.931
	Minimally adj.[d]	1 (Ref)	1.04 (0.70–1.54)	0.94 (0.56–1.57)	1.41 (0.81–2.48)	0.77 (0.34–1.74)	0.805
	Fully adj.[l]	1 (Ref)	0.96 (0.64–1.44)	0.96 (0.57–1.60)	1.52 (0.86–2.70)	0.73 (0.32–1.67)	0.757
TS[j]	Cases (*n*)	282	144	68	51	28	
	Crude	1 (Ref)	0.97 (0.67–1.41)	0.94 (0.58–1.55)	1.29 (0.75–2.21)	1.07 (0.53–2.16)	0.569
	Minimally adj.[d,n]	1 (Ref)	0.99 (0.68–1.45)	0.94 (0.57–1.55)	1.29 (0.74–2.23)	1.18 (0.57–2.45)	0.481
	Fully adj.[l]	1 (Ref)	0.91 (0.62–1.34)	0.94 (0.57–1.56)	1.44 (0.82–2.52)	1.27 (0.61–2.66)	0.319

Abbreviations: Adj., adjusted; CD, cluster of differentiation; Intra-ep, intraepithelial; TS, total score; wk, week.

[a]Odds ratios were calculated per one-level increase in the category of immune cell infiltration (on a 4-level scale) using ordinal logistic regression or when the proportional odds assumption was not met as tested by the Brant test, generalized ordinal logistic regression.

[b]*P* trends were calculated by including physical activity categories (numbered 1–5, lowest to highest) as a continuous variable in the regression models.

[c]Invasive front of the tumor.

[d]Variables included sex, age at diagnosis, and tumor stage.

[e]Variables included sex, age at diagnosis, tumor stage, tumor site, age at baseline, CRP, and year when diagnosed.

[f]Center/core of the tumor.

[g]Within tumor cell nests.

[h]Generalized ordinal logistic regression. Relaxed proportional odds assumption for recreational physical activity.

[i]Generalized ordinal logistic regression. Relaxed proportional odds assumption for physical activity and tumor site.

[j]Calculated as the sum of the scores from each location, ranging from 3 to 12. The total score was classified as low (3–4), intermediate (5–6), or high (7–12).

[k]Generalized ordinal logistic regression. Relaxed proportional odds assumption for recreational physical activity, sex, and age at diagnosis.

[l]Variables included sex, age at diagnosis, tumor stage, tumor site, BMI, smoking, systolic blood pressure, and alcohol intake.

[m]Generalized ordinal logistic regression. Relaxed proportional odds assumption for recreational physical activity and sex.

[n]Generalized ordinal logistic regression. Relaxed proportional odds assumption for sex.

Table 4. Odds ratios (95% confidence intervals) for self-reported, prediagnostic recreational physical activity in relation to tumor immune cell infiltration in colorectal cancer, according to subgroups based on sex, time from baseline to diagnosis, and body size.

	Recreational physical activity					
	Never	**Now and then**	**1–2 times/wk**	**2–3 times/wk**	**>3 times/wk**	***P* trend**[a]
Women[b]						
CD8[c,d]	114	61	32	27	11	
Front[e]	Ref (1.0)	1.31 (0.74–2.32)	0.71 (0.33–1.51)	1.00 (0.47–2.13)	2.15 (0.71–6.55)	0.636
Center[f]	Ref (1.0)	1.59 (0.89–2.84)	0.98 (0.46–2.10)	1.11 (0.52–2.39)	3.53 (1.15–10.2)	0.176
Intra-ep.[g]	Ref (1.0)	1.40 (0.76–2.59)	0.89 (0.41–1.95)	1.27 (0.55–2.93)	0.88 (0.24–3.28)	0.866
TS[h]	Ref (1.0)	1.26 (0.70–2.27)	0.87 (0.42–1.81)	1.20 (0.54–2.66)	2.62 (0.72–9.56)	0.366
CD3[c,d]	135	64	36	27	16	
Front[e]	Ref (1.0)	1.29 (0.74–2.26)	0.73 (0.37–1.42)	1.24 (0.59–2.57)	0.81 (0.32–2.08)	0.808
Center[f]	Ref (1.0)	1.25 (0.73–2.16)	1.15 (0.60–2.21)	1.81 (0.86–3.82)	1.35 (0.50–3.62)	0.169
Intra-ep.[g]	Ref (1.0)	0.92 (0.51–1.64)	0.65 (0.32–1.30)	1.44 (0.67–3.10)	0.50 (0.15–1.59)	0.556
TS[h]	Ref (1.0)	0.92 (0.52–1.64)	0.89 (0.44–1.81)	1.47 (0.66–3.28)	1.28 (0.45–3.59)	0.472
Men[b]						
CD8[c,i]	123	64	23	22	11	
Front[e]	Ref (1.0)	1.40 (0.80–2.45)	1.62 (0.73–3.63)	0.49 (0.20–1.19)	3.95 (1.11–14.1)	0.533
Center[f]	Ref (1.0)	1.22 (0.70–2.14)	1.15 (0.49–2.69)	1.01 (0.42–2.42)	2.26 (0.71–7.23)	0.339
Intra-ep.[g]	Ref (1.0)	0.90 (0.48–1.70)	1.94 (0.82–4.61)	1.27 (0.53–3.07)	1.26 (0.53–4.09)	0.335
TS[h]	Ref (1.0)	1.00 (0.56–1.76)	1.55 (0.67–3.58)	0.78 (0.31–1.99)	1.91 (0.61–5.97)	0.469
CD3[c,i]	147	80	32	24	12	
Front[e]	Ref (1.0)	1.14 (0.68–1.91)	1.24 (0.61–2.51)	0.93 (0.43–2.00)	0.88 (0.29–2.70)	0.986
Center[f]	Ref (1.0)	1.04 (0.63–1.71)	0.90 (0.44–1.87)	1.12 (0.52–2.41)	2.49 (0.91–6.82)	0.257
Intra-ep.[g]	Ref (1.0)	1.13 (0.65–1.95)	1.46 (0.69–3.10)	1.33 (0.57–3.10)	1.20 (0.37–3.89)	0.363
TS[h]	Ref (1.0)	1.01 (0.61–1.69)	0.99 (0.48–2.06)	1.09 (0.50–2.38)	1.18 (0.41–3.39)	0.765
Time from baseline to diagnosis < mean (9.4 years)[b]						
CD8[c,i]	100	57	26	32	17	
Front[e]	Ref (1.0)	1.66 (0.91–3.03)	1.56 (0.68–3.58)	0.81 (0.39–1.68)	2.39 (0.89–6.45)	0.456
Center[f]	Ref (1.0)	1.79 (0.97–3.29)	2.51 (1.10–5.72)	1.43 (0.68–3.02)	3.27 (1.26–8.53)	0.018
Intra-ep.[g]	Ref (1.0)	1.07 (0.56–2.05)	2.08 (0.91–4.77)	1.12 (0.50–2.47)	0.87 (0.30–2.52)	0.705
TS[h]	Ref (1.0)	1.16 (0.63–2.17)	2.12 (0.95–4.72)	1.08 (0.50–2.34)	2.12 (0.80–5.63)	0.149
CD3[c,i]	139	77	40	34	23	
Front[e]	Ref (1.0)	1.05 (0.62–1.77)	0.83 (0.43–1.61)	1.01 (0.53–1.95)	0.67 (0.30–1.52)	0.464
Center[f]	Ref (1.0)	1.08 (0.65–1.79)	0.90 (0.47–1.73)	1.71 (0.87–3.35)	1.50 (0.68–3.30)	0.153
Intra-ep.[g]	Ref (1.0)	0.96 (0.56–1.63)	1.04 (0.53–2.03)	1.03 (0.50–2.12)	0.53 (0.50–1.37)	0.447
TS[h]	Ref (1.0)	0.85 (0.50–1.43)	0.71 (0.36–1.40)	1.11 (0.56–2.20)	0.90 (0.41–2.00)	0.847
Time from baseline to diagnosis ≥mean (9.4 years)[b]						
CD8[c,i]	137	68	29	17	5	
Front[e]	Ref (1.0)	1.17 (0.68–2.00)	0.68 (0.32–1.45)	0.68 (0.26–1.78)	4.14 (0.75–22.7)	0.923
Center[f]	Ref (1.0)	1.14 (0.66–1.94)	0.47 (0.21–1.05)	0.78 (0.30–2.02)	3.92 (0.76–20.2)	0.720
Intra-ep.[g]	Ref (1.0)	1.19 (0.65–2.15)	0.67 (0.29–1.55)	1.55 (0.59–4.07)	1.48 (0.28–7.81)	0.682
TS[h]	Ref (1.0)	1.10 (0.64–1.90)	0.57 (0.26–1.25)	0.95 (0.35–2.58)	4.34 (0.44–42.6)	0.941
CD3[c,i]	143	67	28	17	5	
Front[e]	Ref (1.0)	1.36 (0.80–2.32)	0.99 (0.48–2.05)	1.03 (0.42–2.58)	1.08 (0.20–5.78)	0.804
Center[f]	Ref (1.0)	1.18 (0.69–2.02)	1.14 (0.55–2.39)	0.98 (0.41–2.32)	3.15 (0.61–16.2)	0.158
Intra-ep.[g]	Ref (1.0)	1.06 (0.59–1.92)	0.75 (0.33–1.68)	2.17 (0.86–5.47)	1.08 (0.17–6.76)	0.452
TS[h]	Ref (1.0)	1.10 (0.63–1.92)	1.21 (0.57–2.59)	1.56 (0.60–4.02)	2.53 (0.26–24.8)	0.239
BMI ≥ 25 kg/m² [b]						
CD8[c,i]	166	74	32	29	14	
Front[e]	Ref (1.0)	1.33 (0.80–2.22)	1.43 (0.71–2.87)	1.10 (0.54–2.26)	3.07 (1.10–8.52)	0.086
Center[f]	Ref (1.0)	1.60 (0.96–2.66)	1.65 (0.79–3.43)	1.44 (0.69–3.01)	2.67 (0.97–7.35)	0.028
Intra-ep.[g]	Ref (1.0)	1.12 (0.64–1.95)	1.20 (0.57–2.55)	1.63 (0.76–3.51)	1.02 (0.36–2.90)	0.374
TS[h]	Ref (1.0)	1.12 (0.66–1.88)	1.77 (0.88–3.56)	1.63 (0.76–3.47)	1.94 (0.69–5.46)	0.046
CD3[c,i]	196	85	40	29	19	
Front[e]	Ref (1.0)	1.26 (0.79–2.02)	1.35 (0.73–2.51)	1.28 (0.65–2.53)	0.82 (0.34–1.95)	0.642
Center[f]	Ref (1.0)	1.07 (0.68–1.70)	1.20 (0.64–2.26)	1.59 (0.79–3.19)	1.49 (0.63–3.52)	0.141
Intra-ep.[g]	Ref (1.0)	1.05 (0.64–1.71)	1.09 (0.56–2.10)	1.30 (0.61–2.76)	0.89 (0.35–2.26)	0.770
TS[h]	Ref (1.0)	1.00 (0.62–1.60)	1.11 (0.59–2.11)	1.41 (0.68–2.91)	1.21 (0.50–2.91)	0.385
BMI < 25 kg/m² [b]						
CD8[c,i]	71	51	23	20	8	
Front[e]	Ref (1.0)	1.31 (0.69–2.50)	0.58 (0.23–1.48)	0.40 (0.16–1.03)	2.61 (0.61–11.1)	0.353
Center[f]	Ref (1.0)	1.03 (0.53–2.01)	0.49 (0.20–1.24)	0.59 (0.23–1.51)	3.14 (0.83–11.9)	0.816
Intra-ep.[g]	Ref (1.0)	1.11 (0.54–2.30)	1.10 (0.44–2.76)	0.91 (0.34–2.44)	1.40 (0.29–6.79)	0.876
TS[h]	Ref (1.0)	1.09 (0.55–2.15)	0.49 (0.19–1.25)	0.45 (0.16–1.22)	2.85 (0.64–12.8)	0.457

(Continued on the following page)

Table 4. Odds ratios (95% confidence intervals) for self-reported, prediagnostic recreational physical activity in relation to tumor immune cell infiltration in colorectal cancer, according to subgroups based on sex, time from baseline to diagnosis, and body size. (Cont'd)

	Recreational physical activity					
	Never	**Now and then**	**1–2 times/wk**	**2–3 times/wk**	**>3 times/wk**	***P* trend[a]**
CD3[c,i]	86	59	28	22	9	
Front[e]	Ref (1.0)	1.06 (0.57–1.97)	0.53 (0.24–1.17)	0.80 (0.35–1.85)	0.90 (0.24–3.33)	0.346
Center[f]	Ref (1.0)	1.26 (0.68–2.35)	0.80 (0.37–1.72)	1.20 (0.53–2.76)	2.46 (0.71–8.52)	0.415
Intra-ep.[g]	Ref (1.0)	1.07 (0.55–2.11)	0.76 (0.33–1.73)	1.59 (0.67–3.75)	0.52 (0.09–2.95)	0.920
TS[h]	Ref (1.0)	0.97 (0.51–1.85)	0.71 (0.31–1.61)	1.10 (0.46–2.58)	1.11 (0.30–4.09)	0.970

Abbreviations: BMI, body mass index; CD, cluster of differentiation; Intra-ep, intraepithelial; TS, total score; wk – week.
[a]*P* trends were calculated by including physical activity categories (numbered 1–5, lowest to highest) as a continuous variable in the regression models.
[b]Numbers of observations for total score.
[c]Odds ratios were calculated per one-level increase in the category of immune cell infiltration using ordinal logistic regression.
[d]Covariates included age at diagnosis and tumor stage.
[e]Invasive front of the tumor.
[f]Center/core of the tumor.
[g]Within tumor cell nests.
[h]Calculated as the sum of the scores from each location, ranging from 3 to 12. The total score was classified as low (3–4), intermediate (5–6), or high (7–12).
[i]Covariates included sex, age at diagnosis, and tumor stage.

tissue available. Among these patients, older age, rectal cancer, and higher tumor stage were more common (Supplementary Table S1), consistent with lack of a surgical specimen, which might limit the generalizability of the results. The higher frequency of missing data for $CD8^+$ compared with $CD3^+$ was due to the order of analysis and insufficient tumor tissue volumes remaining after $CD3^+$ IHC was completed. Although this is a potential source of information bias, it seems unlikely to have affected the main results. We have only analyzed immune infiltration of $CD3^+$ and $CD8^+$ in the tumor microenvironment.

We included all cases with prediagnostic data available, including those with baseline during the potentially symptomatic final months prior to diagnosis. Any association between overtly symptomatic cancer and tumor immune infiltration should be adequately accounted for by the multivariable adjustment for tumor site and stage. Also, we do not believe that reduced physical activity due to cancer symptoms contributed to our results, as the expected effect would be a dilution of the risk estimates toward the null. Furthermore, only 13 cases (2.2%) were diagnosed within six months prior to diagnosis, and the analyses stratified by median time from baseline to diagnosis (9.4 years) yielded similar results.

A major strength of our study is the prospectively collected baseline data, which reduces the risk of reverse causality and recall bias. We were also able to account for several potential confounders, including lifestyle-related factors. Although distinguishing between potential confounders and mediators of an association between physical activity and tumor immune cell infiltration is difficult, the consistent results in the crude, minimally adjusted and fully adjusted models suggest that covariate selection did not affect our findings. Furthermore, we used a population-based cohort that gives a representative patient material. Although the tumor sample missingness was not completely at random, a higher total score for the infiltration of both $CD3^+$ and $CD8^+$ immune cells were associated with right-sided colon cancer, female gender, and lower tumor stage (**Table 2**). This is in line with previous studies that have reported that tumor-infiltrating $CD3^+$ and $CD8^+$ immune cells are associated with proximal tumor localization (8, 28, 52–55), female gender (12, 56, 57), and lower tumor stage (8, 9, 28, 55, 58–60), supporting the generalizability of our findings. The use of full tumor tissue sections in our study, though more resource-demanding than tumor tissue microarrays, may also imply a lower risk of sampling error and can, therefore, also be considered a strength of the study. Finally, this is, to our knowledge, the first study to investigate prediagnostic recreational physical activity and tumor immune cell infiltration in colorectal cancer.

Conclusion

In this study based on a population-based cohort, self-reported, prediagnostic recreational physical activity >3 times per week was associated with a higher density of $CD8^+$ immune cells in the front and center of the tumor in colorectal cancer, independent of factors such as age, tumor site, and lifestyle factors. The present study provides some evidence for a potential association between high degree of recreational physical activity and infiltration of $CD8^+$ immune cells in the colorectal cancer tumor microenvironment. However, the results should be interpreted with caution, because there were few observations in the highest degree of physical activity and the use of self-reported physical activity. Thus, a future prospective study with a larger data material is warranted to investigate this association thoroughly.

Authors' Disclosures

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Authors' Contributions

D. Renman: Conceptualization, data curation, formal analysis, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **B. Gylling:** Formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **L. Vidman:** Validation. **S. Bodén:** Data curation, writing–review and editing. **K. Strigård:** Conceptualization, supervision, writing–review and editing. **R. Palmqvist:** Formal analysis, supervision, methodology, writing–review and editing. **S. Harlid:** writing–review and editing. **U. Gunnarsson:** Conceptualization, formal analysis, supervision, validation, project administration, writing–review and editing. **B. van Guelpen:** Conceptualization, formal analysis, supervision, validation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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References

1. Boyle T, Keegel T, Bull F, Heyworth J, Fritschi L. Physical activity and risks of proximal and distal colon cancers: a systematic review and meta-analysis. J Natl Cancer Inst 2012;104:1548–61.
2. Hidayat K, Zhou HJ, Shi BM. Influence of physical activity at a young age and lifetime physical activity on the risks of 3 obesity-related cancers: systematic review and meta-analysis of observational studies. Nutr Rev 2020;78:1–18.
3. Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, et al. Association of leisure-time physical activity with risk of 26 types of cancer in 1.44 million adults. JAMA Intern Med 2016;176:816–25.
4. Wu W, Guo F, Ye J, Li Y, Shi D, Fang D, et al. Pre- and post-diagnosis physical activity is associated with survival benefits of colorectal cancer patients: a systematic review and meta-analysis. Oncotarget 2016;7:52095–103.
5. Je Y, Jeon JY, Giovannucci EL, Meyerhardt JA. Association between physical activity and mortality in colorectal cancer: a meta-analysis of prospective cohort studies. Int J Cancer 2013;133:1905–13.
6. Schmid D, Leitzmann MF. Association between physical activity and mortality among breast cancer and colorectal cancer survivors: a systematic review and meta-analysis. Ann Oncol 2014;25:1293–311.
7. Ballard-Barbash R, Friedenreich CM, Courneya KS, Siddiqi SM, McTiernan A, Alfano CM. Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst 2012;104:815–40.
8. Ling A, Edin S, Wikberg ML, Oberg A, Palmqvist R. The intratumoural subsite and relation of CD8(+) and FOXP3(+) T lymphocytes in colorectal cancer provide important prognostic clues. Br J Cancer 2014;110:2551–9.
9. Dahlin AM, Henriksson ML, Van Guelpen B, Stenling R, Oberg A, Rutegard J, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod Pathol 2011;24:671–82.
10. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol 2014;232:199–209.
11. Mlecnik B, Bindea G, Kirilovsky A, Angell HK, Obenauf AC, Tosolini M, et al. The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis. Sci Transl Med 2016;8:327ra26.
12. Ray AL, Nofchissey RA, Khan MA, Reidy MA, Lerner MR, Wu X, et al. The role of sex in the innate and adaptive immune environment of metastatic colorectal cancer. Br J Cancer 2020;123:624–32.
13. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016;44:698–711.
14. Alexander PG, McMillan DC, Park JH. The local inflammatory response in colorectal cancer – type, location or density? A systematic review and meta-analysis. Cancer Treat Rev 2020;83:101949.
15. Koelwyn GJ, Quail DF, Zhang X, White RM, Jones LW. Exercise-dependent regulation of the tumour microenvironment. Nat Rev Cancer 2017;17:620–32.
16. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. Cell Metab 2016;23:554–62.
17. McClellan JL, Steiner JL, Day SD, Enos RT, Davis MJ, Singh UP, et al. Exercise effects on polyp burden and immune markers in the ApcMin/+ mouse model of intestinal tumorigenesis. Int J Oncol 2014;45:861–8.
18. Campbell JP, Riddell NE, Burns VE, Turner M, van Zanten JJ, Drayson MT, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun 2009;23:767–75.
19. Krüger K, Mooren FC. T cell homing and exercise. Exerc Immunol Rev 2007;13:37–54.
20. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun 2011;25:1521–9.
21. Bacurau AV, Belmonte MA, Navarro F, Moraes MR, Pontes FL Jr, Pesquero JL, et al. Effect of a high-intensity exercise training on the metabolism and function of macrophages and lymphocytes of walker 256 tumor bearing rats. Exp Biol Med 2007;232:1289–99.
22. Hagar A, Wang Z, Koyama S, Serrano JA, Melo L, Vargas S, et al. Endurance training slows breast tumor growth in mice by suppressing Treg cells recruitment to tumors. BMC Cancer 2019;19:536.
23. Rundqvist H, Veliça P, Barbieri L, Gameiro PA, Bargiela D, Gojkovic M, et al. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. Elife 2020;9:e59996.
24. Jones S, Chen WD, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, et al. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 2008;105:4283–8.
25. Norberg M, Wall S, Boman K, Weinehall L. The Västerbotten Intervention Programme: background, design and implications. Glob Health Action 2010;3.
26. Peters T, Brage S, Westgate K, Franks PW, Gradmark A, Tormo Diaz MJ, et al. Validity of a short questionnaire to assess physical activity in 10 European countries. Eur J Epidemiol 2012;27:15–25.
27. Johansson I, Hallmans G, Wikman A, Biessy C, Riboli E, Kaaks R. Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort. Public Health Nutr 2002;5:487–96.
28. Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res 2009;15:6412–20.
29. Williams R. Understanding and interpreting generalized ordered logit models. J Math Sociol 2016;40:7–20.
30. Carr PR, Alwers E, Bienert S, Weberpals J, Kloor M, Brenner H, et al. Lifestyle factors and risk of sporadic colorectal cancer by microsatellite instability status: a systematic review and meta-analyses. Ann Oncol 2018;29:825–34.
31. Hughes LA, Simons CC, van den Brandt PA, Goldbohm RA, de Goeij AF, de Bruïne AP, et al. Body size, physical activity and risk of colorectal cancer with or without the CpG island methylator phenotype (CIMP). PLoS One 2011;6:e18571.
32. Jayasekara H, English DR, Haydon A, Hodge AM, Lynch BM, Rosty C, et al. Associations of alcohol intake, smoking, physical activity and obesity with survival following colorectal cancer diagnosis by stage, anatomic site and tumor molecular subtype. Int J Cancer 2018;142:238–50.
33. Hardikar S, Newcomb PA, Campbell PT, Win AK, Lindor NM, Buchanan DD, et al. Prediagnostic physical activity and colorectal cancer survival: overall and stratified by tumor characteristics. Cancer Epidemiol Biomarkers Prev 2015;24:1130–7.
34. Bliss R, Weinberg J, Webster T, Vieira V. Determining the probability distribution and evaluating sensitivity and false positive rate of a confounder detection method applied to logistic regression. J Biom Biostat 2012;3:142.
35. Martinez-Useros J, Garcia-Foncillas J. Obesity and colorectal cancer: molecular features of adipose tissue. J Transl Med 2016;14:21.
36. Chang ML, Yang Z, Yang SS. Roles of adipokines in digestive diseases: markers of inflammation, metabolic alteration and disease progression. Int J Mol Sci 2020;21:8308.
37. Koh H, Hamada T, Song M, Liu L, Cao Y, Nowak JA, et al. Physical activity and colorectal cancer prognosis according to tumor-infiltrating T cells. JNCI Cancer Spectr 2018;2:pky058.

38. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997;186:1407–18.
39. Martin MD, Badovinac VP. Defining memory CD8 T cell. Front Immunol 2018; 9:2692.
40. Arem H, Pfeiffer RM, Engels EA, Alfano CM, Hollenbeck A, Park Y, et al. Pre- and postdiagnosis physical activity, television viewing, and mortality among patients with colorectal cancer in the National Institutes of Health-AARP Diet and Health Study. J Clin Oncol 2015;33:180–8.
41. Ratjen I, Schafmayer C, di Giuseppe R, Waniek S, Plachta-Danielzik S, Koch M, et al. Postdiagnostic physical activity, sleep duration, and TV watching and all-cause mortality among long-term colorectal cancer survivors: a prospective cohort study. BMC Cancer 2017;17:701.
42. Hong J, Park J. Systematic review: recommendations of levels of physical activity among colorectal cancer patients (2010–2019). Int J Environ Res Public Health 2021;18:2896.
43. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol 2019;14:89–103.
44. Abar L, Vieira AR, Aune D, Sobiecki JG, Vingeliene S, Polemiti E, et al. Height and body fatness and colorectal cancer risk: an update of the WCRF-AICR systematic review of published prospective studies. Eur J Nutr 2018;57:1701–20.
45. Hanyuda A, Ogino S, Qian ZR, Nishihara R, Song M, Mima K, et al. Body mass index and risk of colorectal cancer according to tumor lymphocytic infiltrate. Int J Cancer 2016;139:854–68.
46. Berntsson J, Eberhard J, Nodin B, Leandersson K, Larsson AH, Jirström K. Pre-diagnostic anthropometry, sex, and risk of colorectal cancer according to tumor immune cell composition. Oncoimmunology 2019;8:e1664275.
47. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Act 2008;5:56.
48. Schuna JM Jr, Johnson WD, Tudor-Locke C. Adult self-reported and objectively monitored physical activity and sedentary behavior: NHANES 2005–2006. Int J Behav Nutr Phys Act 2013;10:126.
49. Dyrstad SM, Hansen BH, Holme IM, Anderssen SA. Comparison of self-reported versus accelerometer-measured physical activity. Med Sci Sports Exerc 2014;46:99–106.
50. Prince SA, Roberts KC, Reed JL, Biswas A, Colley RC, Thompson W. Daily physical activity and sedentary behaviour across occupational classifications in Canadian adults. Health Rep 2020;31:13–26.
51. Steeves JA, Tudor-Locke C, Murphy RA, King GA, Fitzhugh EC, Bassett DR, et al. Daily physical activity by occupational classification in US adults: NHANES 2005–2006. J Phys Act Health 2018:1–12.
52. Prizment AE, Vierkant RA, Smyrk TC, Tillmans LS, Nelson HH, Lynch CF, et al. Cytotoxic T cells and granzyme B associated with improved colorectal cancer survival in a prospective cohort of older women. Cancer Epidemiol Biomarkers Prev 2017;26:622–31.
53. Schweiger T, Berghoff AS, Glogner C, Glueck O, Rajky O, Traxler D, et al. Tumor-infiltrating lymphocyte subsets and tertiary lymphoid structures in pulmonary metastases from colorectal cancer. Clin Exp Metastasis 2016;33:727–39.
54. Yoon HH, Orrock JM, Foster NR, Sargent DJ, Smyrk TC, Sinicrope FA. Prognostic impact of FoxP3+ regulatory T cells in relation to CD8+ T lymphocyte density in human colon carcinomas. PLoS One 2012;7:e42274.
55. Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2009;27:186–92.
56. Nearchou IP, Lillard K, Gavriel CG, Ueno H, Harrison DJ, Caie PD. Automated analysis of lymphocytic infiltration, tumor budding, and their spatial relationship improves prognostic accuracy in colorectal cancer. Cancer Immunol Res 2019;7:609–20.
57. McCoy MJ, Hemmings C, Anyaegbu CC, Austin SJ, Lee-Pullen TF, Miller TJ, et al. Tumour-infiltrating regulatory T cell density before neoadjuvant chemoradiotherapy for rectal cancer does not predict treatment response. Oncotarget 2017;8:19803–13.
58. Berntsson J, Svensson MC, Leandersson K, Nodin B, Micke P, Larsson AH, et al. The clinical impact of tumour-infiltrating lymphocytes in colorectal cancer differs by anatomical subsite: a cohort study. Int J Cancer 2017;141:1654–66.
59. Richards CH, Roxburgh CS, Powell AG, Foulis AK, Horgan PG, McMillan DC. The clinical utility of the local inflammatory response in colorectal cancer. Eur J Cancer 2014;50:309–19.
60. Nosho K, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol 2010;222:350–66.