EUROPEAN UROLOGY 76 (2019) 33–40

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Prostate Cancer

Editorial by Ola Bratt and Alastair D. Lamb on pp. 41–42 of this issue

A Prospective Study of the Association between Physical Activity and Risk of Prostate Cancer Defined by Clinical Features and *TMPRSS2:ERG*

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Article info

Article history:
Accepted September 21, 2018

Associate Editor:
Giacomo Novara

Keywords:
Exercise
Molecular biomarkers
Physical activity
Prevention
Prostate cancer

Abstract

Background: Growing evidence shows that clinical and molecular subtypes of prostate cancer (PCa) have specific risk factors. Observational studies suggest that physical activity may lower the risk of aggressive PCa. To our knowledge, the association between physical activity and PCa defined by *TMPRSS2:ERG* has not been evaluated.
Objective: To prospectively examine the association between physical activity and risk of PCa defined by clinical features and *TMPRSS2:ERG*.
Design, setting, and participants: We studied 49 160 men aged 40–75 yr in the Health Professionals Follow-up Study from 1986 to 2012. Data was collected at baseline and every 2 yr with >90% follow-up. Total and vigorous physical activity were measured in metabolic equivalent of task (MET)-h/wk.
Outcome measures and statistical analysis: Advanced PCa was defined as stage T3b, T4, N1, or M1 at diagnosis and lethal PCa as distant metastases or death due to disease over follow-up. Presence of *TMPRSS2:ERG* was estimated by immunohistochemistry of ERG protein expression. Cox proportional hazards models were used to obtain multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) for incidence of subtype-specific PCa.
Results and limitations: During 26 yr of follow-up, 6411 developed PCa overall and 888 developed lethal disease. There were no significant associations between total physical activity and risk of PCa in the overall cohort. In multivariable-adjusted models, men in the highest quintile of vigorous activity had a significant 30% lower risk of

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https://doi.org/10.1016/j.eururo.2018.09.041

advanced PCa (HR: 0.70, 95% CI: 0.53–0.92) and 25% lower risk of lethal PCa (HR: 0.75, 95% CI: 0.59–0.94) than men in the lowest quintile of vigorous activity. The association was independent of screening history. Vigorous activity was not associated with total PCa in the overall cohort but was inversely associated among highly screened men (top vs bottom quintile, HR: 0.83, 95% CI: 0.70–0.97). Of all cases, 945 were assayed for ERG (48% ERG-positive). Men with higher vigorous activity had a lower risk of ERG-positive PCa (top vs bottom quintile, HR: 0.71, 95% CI: 0.52–0.97). There was no significant association with the risk of ERG-negative disease (p heterogeneity = 0.09).
Conclusions: Our study confirms that vigorous physical activity is associated with lower risk of advanced and lethal PCa and provides novel evidence for a lower risk of *TMPRSS2:ERG*-positive disease.
Patient summary: The identification of modifiable lifestyle factors for prevention of clinically important prostate cancer (PCa) is needed. In this report, we compared risk of PCa in men with different levels of physical activity. Men with higher vigorous activity had a lower risk of developing advanced and lethal PCa and PCa with the common *TMPRSS2:ERG* gene fusion.

1. Introduction

Given the large burden of prostate cancer (PCa) globally, modifiable lifestyle factors that could lower a man's risk of PCa must be identified. Epidemiologic studies of the relationship between physical activity and risk of PCa overall have been mixed but suggest a moderate inverse association [1]. In some epidemiologic studies, men who engaged in higher levels of physical activity had lower risks of developing advanced and fatal PCa [2–5]. However, other studies have shown no significant association between physical activity and advanced or fatal disease [6–8]. Physical activity influences a wide range of biological processes, including hormonal, anti-inflammatory, and insulin pathways [9,10]. These pathways are implicated in the development of aggressive PCa, suggesting a link between physical activity and clinically relevant disease [11].

The integration of PCa characteristics related to not only clinical course [2] but also molecular features [12–14] in epidemiologic studies may be the key to understanding the relationship between physical activity and PCa risk. The *TMPRSS2:ERG* gene fusion is the most common PCa molecular subtype [12]. Found in 40–50% of primary PCas, *TMPRSS2:ERG* results in androgen-regulated expression of the oncogene *ERG* [15]. Androgens, cellular stress, and insulin-like growth factor (IGF) signaling may have a role in the development and progression of fusion-positive cancers [16–18]. Although *TMPRSS2:ERG* does not independently predict biochemical recurrence or lethal disease [19], the fusion may interact with risk factors to influence PCa prognosis [14,18]. Furthermore, some PCa risk factors, such as low tomato sauce intake and taller height, are more strongly associated with the development of fusion-positive versus fusion-negative PCa [20–22]. In this study, we hypothesized that the influence of physical activity on hormonal and anti-inflammatory pathways protects against the development of fusion-positive PCa.

The objective of this study was to examine the associations between long-term, pre-diagnostic physical activity among men and risk of developing PCa defined by clinical features (stage, grade, and lethality) and molecular (*TMPRSS2:ERG*) subtype.

2. Materials and methods

2.1. Study population

The Health Professionals Follow-up Study (HPFS) is an ongoing prospective cohort initiated in 1986 among 51 529 US male health professionals aged 40–75 yr at baseline. Participants completed biennial questionnaires beginning at baseline to ascertain lifestyle, health-related factors, and disease outcomes. Usual diet was assessed every 4 yr using a validated food frequency questionnaire. Follow-up exceeded 90% in each cycle. The study population for this analysis consisted of 49 160 men. We excluded men who reported cancers except non-melanoma skin cancer prior to baseline (n = 2087) and those with missing date of birth (n = 32) or baseline physical activity (n = 250). The study was approved by the Human Subjects Research Committee at the Harvard T.H. Chan School of Public Health.

2.2. Assessment of physical activity

Physical activity was assessed through biennial, validated questionnaires [23] beginning at baseline. Participants selected a category for the average total time/wk engaged in specific activities during the past year: walking or hiking outdoors, jogging, running, bicycling, lap swimming, tennis, squash or racquetball, and calisthenics or rowing. Participants also reported their usual walking pace and the number of flights of stairs climbed daily. Additional specific activities were included on the questionnaire in subsequent cycles: heavy outdoor work from 1988, weightlifting from 1990, moderate outdoor work from 2004, and lower intensity exercise and other aerobic exercise from 2010. Participants indicated the intensity of activity (low, medium, high) for swimming, biking, and tennis from 2010.

To quantify activity intensity, each activity was assigned a metabolic equivalent of task (MET) value based on a compendium of physical activities [24]. A unit of MET is equal to the amount of oxygen uptake required to sit at rest, approximately 3.5 ml/kg/min. A MET-hour is the metabolic equivalent of sitting at rest for 1 h. A measure of MET-h/wk was derived for each activity by multiplying the activity-specific MET value by the participant-reported average number of h/wk. Total activity was defined as the sum of MET-h/wk for each activity. Vigorous activity included activities with a MET value $\geq$6: jogging, running, bicycling, lap swimming, tennis, squash/racquetball, calisthenics/rowing, and stair climbing.

2.3. Ascertainment of PCa outcomes

Incident PCa was captured by self-report and confirmed through medical records and pathology reports. Information on clinical and treatment

Table 1 – Age-adjusted characteristics by quintile of total and vigorous physical activity (MET-h/wk) among men in the Health Professionals Follow-up Study at baseline in 1986 unless otherwise specified

Characteristics	Total activity quintile			Vigorous activity quintile		
	Q1	Q3	Q5	Q1	Q3	Q5
Participants, *n*	9801	9646	9935	8888	9734	9989
Age, mean (SD), yr [a]	54.9 (9.7)	54.5 (9.7)	53.3 (9.7)	57.2 (9.7)	54.6 (9.8)	51.1 (8.8)
Total activity, mean (SD), MET-h/wk	0.8 (0.7)	10.2 (2.2)	53.7 (36.4)	7.6 (12.3)	9.0 (10.9)	46.8 (40.1)
Vigorous activity, mean (SD), MET-h/wk	0.2 (0.4)	5.2 (4.3)	33.1 (38.4)	0.0 (0.0)	2.4 (1.3)	38.7 (36.0)
PSA screening history						
Had PSA test in 1994, %	34	39	40	35	37	42
No. of biennial questionnaires with PSA test, 1994–2010	5.2	5.6	5.6	5.2	5.6	5.8
PSA test on at least half of all questionnaires, 1994–2010, %	62	69	68	62	68	70
Family history of prostate cancer, %	11	12	12	11	12	12
Diabetes, %	4.3	2.8	2.3	4.2	3.3	2.1
Caucasian, %	95	96	96	95	96	95
Current smokers, %	15	9.4	6.9	15	9.5	4.9
Multivitamin use, %	38	44	46	37	44	49
Height, mean (SD), inches	70.0 (2.9)	70.1 (2.8)	70.2 (2.9)	70.1 (3.0)	70.1 (2.9)	70.1 (2.8)
BMI at age 21 yr, mean (SD), kg/m^2	22.9 (3.2)	23.0 (3.0)	23.2 (2.9)	23.0 (3.3)	23.0 (3.0)	23.1 (2.7)
BMI, mean (SD), kg/m^2	26.2 (3.8)	25.5 (3.3)	24.8 (3.0)	26.2 (3.7)	25.7 (3.4)	24.7 (2.8)
Dietary & nutrient intakes, mean (SD)						
Total calories, kcal/d	1936 (621)	1969 (609)	2053 (635)	1954 (626)	2002 (627)	2011 (613)
Calcium, mg/d	861 (427)	900 (419)	926 (434)	862 (429)	899 (417)	946 (449)
α-linolenic acid, g/d	1.1 (0.4)	1.1 (0.4)	1.0 (0.3)	1.1 (0.4)	1.1 (0.4)	1.0 (0.3)
Supplemental vitamin E, mg/d	31.5 (79.1)	38.4 (84.2)	44.8 (91.7)	32.2 (80.5)	37.7 (83.1)	48.8 (94.6)
Tomato sauce, servings/wk	0.9 (1.3)	0.9 (1.1)	1.0 (1.3)	0.9 (1.2)	1.0 (1.2)	1.0 (1.2)
Alcohol, g/d	10.8 (16.3)	11.1 (14.9)	12.3 (15.5)	11.2 (16.8)	10.7 (14.6)	11.4 (14.4)
Coffee, cups/d	2.0 (1.9)	1.9 (1.8)	1.8 (1.7)	2.0 (1.9)	1.9 (1.8)	1.8 (1.7)

BMI = body mass index; PSA = prostate-specific antigen.
[a] Variable not adjusted for age.

history and disease progression was collected through medical records as well as biennial disease-specific questionnaires for development of metastases. Deaths were ascertained through repeated mailings, telephone calls to non-respondents, and searches of the National Death Index. An endpoint committee of physicians confirmed PCa-specific death.

We classified clinical subgroups of PCa as follows: (1) localized PCa: stage T1 or T2 and N0, M0 at diagnosis; (2) advanced PCa: stage T3b, T4, N1, or M1 at diagnosis; and (3) lethal PCa: distant metastases or PCa death over follow-up. PCa cases were also defined as high-grade (Gleason 8–10 and 4 + 3) or low-grade (Gleason 2–6 and 3 + 4). Stage T1a cases ($n = 286$) were excluded since these cases are incidentally diagnosed and prone to detection bias.

Tumor tissue microarrays were constructed using archival formalin-fixed paraffin-embedded prostate tumor tissue from radical prostatectomy (RP) or transurethral resection of the prostate (TURP) as previously described [19]. Presence or absence of the *TMPRSS2:ERG* fusion was assessed using a validated immunohistochemistry assay for ERG protein expression. This study included 910 RP and 35 TURP specimens assayed for ERG, diagnosed from 1986 to 2009.

2.4. Statistical analysis

Each participant contributed person-time from date of return of the baseline questionnaire to date of PCa diagnosis, death, or end of follow-up (January 31, 2012). For ERG-defined PCa outcomes, follow-up ended on December 31, 2009 because this was the last year a case assayed for ERG was diagnosed. Cox proportional hazards regression was used to estimate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) between physical activity (by quintile) and incidence of total, lethal, advanced, localized, high-grade, and low-grade PCa. An extension of Cox proportional hazards regression was used to model the associations between physical activity and PCa incidence according to ERG subtype [25,26]. For ERG-specific PCa outcomes, additional analyses of the 910 RP cases assayed for ERG were performed, applying inverse probability weights to account for clinical factors at diagnosis among cases [21]. Tests for heterogeneity of these HRs across quintiles were performed using a likelihood ratio test [26].

To examine long-term activity, we used cumulative average physical activity updated every 2 yr from baseline in 1986 to the time of PCa diagnosis, death, or end of follow-up. The cumulative average physical activity was categorized into quintiles based on the distribution in each questionnaire cycle.

Age- and multivariable-adjusted models included age in months and calendar time. Only multivariable models are presented because the results were similar to age-adjusted models. Multivariable models were additionally adjusted for race, family history of PCa, diabetes, body mass index (BMI), height, smoking status, multivitamin use, and dietary factors. All variables except for race, family history, and BMI at age 21 yr were updated over follow-up. All models of vigorous activity were additionally adjusted for nonvigorous activity. Nonvigorous activity was allowed to vary by ERG subtype in models for ERG-defined PCa.

To account for potential detection bias, we adjusted for having had a prostate-specific antigen (PSA) test, lagged by one cycle to better capture screening rather than diagnostic PSA tests, and PSA testing intensity over time (defined as reporting a PSA test in half or more questionnaire cycles since 1994). To address potential residual confounding by PSA testing, we conducted the analysis among a highly screened subcohort of

Table 2 – Hazard ratios and 95% confidence intervals for the association of total physical activity (MET-h/wk) and risk of prostate cancer overall and by clinical subgroup [a] in the total Health Professional Follow-up Study cohort with follow-up from 1986 to 2012 and in the highly screened subcohort with follow-up from 1996 to 2012

	Total cohort, 1986–2012				Highly screened subcohort, 1996–2012 [b]			
	Total activity quintile, HR (95% CI)				Total activity quintile, HR (95% CI)			
	Q1	Q3	Q5	p_{trend}	Q1	Q3	Q5	p_{trend}
Total prostate cancer								
No. incident cases	1270	1275	1252		316	361	354	
Multivariable [c]	1 (Ref)	1.00 (0.92–1.08)	0.98 (0.91–1.07)	0.8	1 (Ref)	0.96 (0.82–1.12)	0.92 (0.79–1.09)	0.2
Lethal prostate cancer								
No. incident cases	190	174	166		25	31	28	
Multivariable [c]	1 (Ref)	0.95 (0.77–1.18)	0.95 (0.76–1.18)	0.5	1 (Ref)	1.04 (0.60–1.82)	1.12 (0.63–1.98)	0.9
Advanced prostate cancer								
No. incident cases	123	107	117		17	15	20	
Multivariable [c]	1 (Ref)	0.91 (0.70–1.18)	1.00 (0.77–1.30)	0.9	1 (Ref)	0.74 (0.36–1.53)	0.89 (0.44–1.79)	0.7
Localized prostate cancer								
No. incident cases	882	971	911		253	297	285	
Multivariable [c]	1 (Ref)	1.06 (0.97–1.17)	0.99 (0.90–1.09)	0.8	1 (Ref)	0.97 (0.82–1.16)	0.92 (0.77–1.10)	0.2
High-grade prostate cancer								
No. incident cases	251	269	280		62	72	77	
Multivariable [c]	1 (Ref)	1.09 (0.92–1.30)	1.14 (0.96–1.37)	0.2	1 (Ref)	1.08 (0.76–1.53)	1.15 (0.80–1.63)	0.8
Low-grade prostate cancer								
No. incident cases	728	809	778		207	252	237	
Multivariable [c]	1 (Ref)	1.05 (0.95–1.16)	1.00 (0.90–1.11)	1	1 (Ref)	0.99 (0.82–1.20)	0.92 (0.75–1.11)	0.2

CI = confidence interval; HR = hazard ratio; PSA = prostate-specific antigen.

[a] Lethal prostate cancer: distant metastasis or death due to the disease; advanced prostate cancer: stage T3b, T4, N1, or M1 at diagnosis; localized prostate cancer: stage T1 or T2 and N0, M0 at diagnosis; high-grade: Gleason 8–10 and 4 + 3; low-grade: Gleason 2–6 and 3 + 4.

[b] Highly screened subcohort of 13 859 men who reported having a PSA test on the 1994 and 1996 questionnaires, with follow-up starting in 1996.

[c] Multivariable models in the total cohort are adjusted for age (mo), calendar time (mo), height (in; ≤68, >68 to 70, >70 to 72, >72), race (Caucasian or other), family history of prostate cancer in father or brother (yes or no), BMI at age 21 yr (kg/m^2; ≤20, 21 to <23, 23 to <25, ≥25), intensity of prostate-specific antigen (PSA) testing (yes or no), having a PSA test in previous cycle (yes or no), smoking (never, former/quit >10 yr ago, former/quit ≤10 yr ago, or current), diabetes (yes or no), current BMI (kg/m^2; ≤22, 23 to <25, 25 to <27.5, ≥27.5), multivitamin use (yes or no), intake total calories (quintiles), calcium (quintiles), tomato sauce (servings/wk; <0.25, 0.25 to <1.0, 1.0 to <2.0, ≥2), α-linolenic acid (quintiles), alcohol (quintiles), coffee (cups/d; 0, >0 to 1, >1 to 2, >2 to 3, >3), and vitamin E supplements (quintiles); multivariable models in highly screened cohort are adjusted for those listed except for height, BMI at age 21 yr, tomato sauce, α-linolenic acid, and having a PSA test in previous cycle.

13 859 men who reported having a PSA test on the 1994 and 1996 questionnaires, with follow-up starting in 1996 [27].

These analyses were conducted using SAS version 9.4 (SAS Institute Inc.; Cary, NC, USA). All statistical tests were two-sided with an α level of 0.05 to determine statistical significance.

3. Results

Table 1 shows the age-adjusted characteristics of the 49 160 men at baseline in 1986, according to quintiles of total and vigorous physical activity. The median amount of total activity was 10.2 MET-h/wk and vigorous was 2.2 MET-h/wk. Men in higher quintiles of physical activity tended to be younger, have lower BMI, were more likely to be nonsmokers and use multivitamins, and reported more PSA testing between 1994 and 2010 than men in lower quintiles.

Between 1986 and 2012, 6411 men were diagnosed with incident PCa (Supplementary Table 1) including 603 with advanced and 888 with lethal disease. Of 945 cases assayed for ERG, 449 (48%) had ERG-positive disease.

Tables 2 and 3 show results from multivariable-adjusted models for the associations of total activity and vigorous activity with the risk of PCa defined by clinical features in the total cohort and in the highly screened subcohort. There was no association between total activity and risk of PCa overall or of any clinical subgroup in either cohort. In contrast, men in the highest quintile of vigorous activity had a significant 30% lower risk of advanced PCa (top vs bottom quintile, HR: 0.70; 95% CI: 0.53–0.92; p trend = 0.04) and a 25% lower risk of lethal PCa (top vs bottom quintile, HR: 0.75; 95% CI: 0.59–0.94; p trend = 0.04) than men in the lowest quintile in the total cohort. Additionally, there was a borderline significant 16% lower risk of high-grade PCa in the highest than in the lowest quintile of vigorous activity (HR: 0.84; 95% CI: 0.70–1.01). Vigorous activity was not significantly associated with the risk of overall, localized, or low-grade PCa in multivariable-adjusted models in the total cohort. After restricting to highly screened men, however, vigorous activity was associated with a 16–18% lower risk of total, localized, and low-grade PCa.

Table 4 presents associations of total and vigorous activity with risk of ERG-defined PCa. As with clinical features, we did not observe significant associations between total activity and PCa risk of either ERG subtype. However, for vigorous activity, men in the highest quintile had a significant 29% lower risk of ERG-positive PCa than men in the lowest quintile (top vs bottom quintile, HR: 0.71, 95% CI: 0.52–0.97; p trend = 0.04). There was no significant association between vigorous activity and risk of ERG-negative PCa (p heterogeneity = 0.09). After restricting to the highly screened subcohort, the association between vigorous activity and ERG-positive disease persisted, and

Table 3 – Hazard ratios and 95% confidence intervals for the association of vigorous physical activity (MET-h/wk) and risk of prostate cancer overall and by clinical subgroup [a] in the total Health Professionals Follow-up Study cohort with follow-up from 1986 to 2012 and in the highly screened subcohort with follow-up from 1996 to 2012

	Total cohort, 1986–2012				Highly screened subcohort, 1996–2012 [b]			
	Vigorous activity quintile, HR (95% CI)				Vigorous activity quintile, HR (95% CI)			
	Q1	Q3	Q5	p_{trend}	Q1	Q3	Q5	p_{trend}
Total prostate cancer								
No. incident cases	1405	1319	1129		337	375	327	
Multivariable [c]	1 (Ref)	1.00 (0.92–1.08)	0.95 (0.88–1.04)	0.3	1 (Ref)	0.97 (0.83–1.13)	0.83 (0.70–0.97)	0.02
Lethal prostate cancer								
No. incident cases	248	182	109		37	39	23	
Multivariable [c]	1 (Ref)	0.90 (0.74–1.09)	0.75 (0.59–0.94)	0.04	1 (Ref)	1.01 (0.62–1.63)	0.82 (0.47–1.44)	0.8
Advanced prostate cancer								
No. incident cases	167	116	80		17	11	15	
Multivariable [c]	1 (Ref)	0.82 (0.64–1.05)	0.70 (0.53–0.92)	0.04	1 (Ref)	0.54 (0.24–1.19)	0.73 (0.34–1.57)	0.4
Localized prostate cancer								
No. incident cases	975	982	864		269	307	263	
Multivariable [c]	1 (Ref)	1.04 (0.94–1.13)	0.99 (0.89–1.09)	0.7	1 (Ref)	0.98 (0.83–1.16)	0.82 (0.68–0.98)	0.04
High-grade prostate cancer								
No. incident cases	341	292	232		82	89	61	
Multivariable [c]	1 (Ref)	0.94 (0.80–1.10)	0.84 (0.70–1.01)	0.3	1 (Ref)	0.99 (0.72–1.35)	0.70 (0.49–1.00)	0.13
Low-grade prostate cancer								
No. incident cases	764	787	743		212	242	224	
Multivariable [c]	1 (Ref)	1.03 (0.93–1.14)	1.01 (0.91–1.13)	0.8	1 (Ref)	0.97 (0.80–1.18)	0.84 (0.69–1.03)	0.03

CI = confidence interval; HR = hazard ratio; PSA = prostate-specific antigen.

[a] Lethal prostate cancer: distant metastasis or death due to the disease; advanced prostate cancer: stage T3b, T4, N1, or M1 at diagnosis; localized prostate cancer: stage T1 or T2 and N0, M0 at diagnosis; high-grade: Gleason 8–10 and 4 + 3; low-grade: Gleason 2–6 and 3 + 4.

[b] Highly screened subcohort of 13 859 men who reported having a PSA test on the 1994 and 1996 questionnaires, with follow-up starting in 1996.

[c] Multivariable models in the total cohort are adjusted for age (mo), calendar time (mo), non-vigorous activity (quintiles), height (in; ≤68, >68 to 70, >70 to 72, >72), race (Caucasian or other), family history of prostate cancer in father or brother (yes or no), BMI at age 21 yr (kg/m^2; ≤20, 21 to <23, 23 to <25, ≥25), intensity of prostate-specific antigen (PSA) testing (yes or no), having a PSA test in previous cycle (yes or no), smoking (never, former/quit >10 yr ago, former/quit ≤10 yr ago, or current), diabetes (yes or no), current BMI (kg/m^2; ≤22, 23 to <25, 25 to <27.5, ≥27.5), multivitamin use (yes or no), intake total calories (quintiles), calcium (quintiles), tomato sauce (servings/wk; <0.25, 0.25 to <1.0, 1.0 to <2.0, ≥2), α-linolenic acid (quintiles), alcohol (quintiles), coffee (cups/d; 0, >0 to 1, >1 to 2, >2 to 3, >3), and vitamin E supplements (quintiles); multivariable models in highly screened cohort are adjusted for those listed except for height, BMI at age 21 yr, tomato sauce, α-linolenic acid, and having a PSA test in previous cycle.

Table 4 – Hazard ratios and 95% confidence intervals for the association of total and vigorous physical activity (MET-h/wk) quintiles and risk of ERG-positive and ERG-negative prostate cancer in the Health Professionals Follow-up Study with follow-up from 1986 to 2009

	ERG-negative		ERG-positive		
	No. of cases	Multivariable [a] HR (95% CI)	No. of cases	Multivariable [a] HR (95% CI)	$p_{heterogeneity}$
Total activity quintile					0.2[c]
Q1	97	1.00 (ref)	69	1.00 (ref)	
Q2	83	0.84 (0.62–1.12)	84	1.19 (0.86–1.64)	
Q3	113	1.12 (0.85–1.48)	102	1.45 (1.06–1.97)	
Q4	97	0.95 (0.71–1.26)	111	1.52 (1.12–2.06)	
Q5	106	1.04 (0.78–1.38)	83	1.13 (0.82–1.57)	
p_{trend}		0.6		0.6	1[d]
Vigorous activity quintile [b]					0.5[c]
Q1	98	1.00 (ref)	103	1.00 (ref)	
Q2	95	0.99 (0.74–1.32)	89	0.84 (0.63–1.13)	
Q3	106	1.08 (0.82–1.44)	94	0.89 (0.67–1.19)	
Q4	97	0.98 (0.73–1.30)	86	0.78 (0.58–1.05)	
Q5	100	1.05 (0.78–1.40)	77	0.71 (0.52–0.97)	
P_{trend}		0.8		0.04	0.09[d]

CI = confidence interval; HR = hazard ratio; PSA = prostate-specific antigen.

[a] Multivariable models adjusted for age (mo), calendar time (mo), height (in; ≤68, >68 to 70, >70 to 72, >72), race (Caucasian or other), family history of prostate cancer in father or brother (yes or no), BMI at age 21 (kg/m^2; ≤20, 21 to <23, 23 to <25, ≥25), intensity of prostate-specific antigen (PSA) testing (yes or no), having a PSA test in previous cycle (yes or no), smoking (never, former/quit >10 yr ago, former/quit ≤10 yr ago, or current), diabetes (yes or no), current BMI (kg/m^2; ≤22, 23 to <25, 25 to <27.5, ≥27.5), multivitamin use (yes or no), intake total calories (quintiles), calcium (quintiles), tomato sauce (servings/week; <0.25, 0.25 to <1.0, 1.0 to <2.0, ≥2), α-linolenic acid (quintiles), alcohol (quintiles), coffee (cups/d; 0, >0 to 1, >1 to 2, >2 to 3, >3), and vitamin E supplements (quintiles).

[b] Multivariable models with vigorous activity (quintiles) are additionally adjusted for non-vigorous activity (quintiles).

[c] Based on a likelihood ratio test with four degrees of freedom using quintiles as the exposure.

[d] Based on a likelihood ratio test with one degree of freedom using continuous trend variable as the exposure.

there was a suggestive inverse association between total activity and ERG-positive disease (Supplementary Table 2). The association between vigorous activity and ERG-positive PCa was similar in magnitude when restricting to RP cases and when using inverse probability weighting to account for potential differences among cases with and without tissue biomarker data (data not shown).

4. Discussion

Our prospective cohort analysis supports a moderate inverse association between long-term physical activity of vigorous intensity and risk of developing advanced and lethal PCa. Furthermore, our findings suggest that vigorous activity is associated with a lower risk of *TMPRSS2:ERG*-positive PCa. To our knowledge, this is the first study to examine physical activity and *TMPRSS2:ERG*-defined PCa.

As a modifiable risk factor, physical activity is an important target for public health intervention. Our findings suggest that the relationship between physical activity and PCa risk may not be linear, with potential benefits observed primarily in the upper quintile of vigorous activity. In our study, men in the lowest quintile engaged in 1 MET-h/wk or less of vigorous activity, whereas men in the highest quintile performed a median of 30 MET-h/wk of vigorous activity, equivalent to approximately 25 min/d of running, which is two times the nationally recommended amount of weekly vigorous-intensity activity [28]. The finding that physical activity is potentially beneficial with respect to clinically relevant PCa augments existing evidence that men should increase their physical activity, even before receiving a cancer diagnosis.

Our results are consistent with previously reported inverse associations between physical activity and aggressive PCa phenotypes [2–5,29]. In the Cancer Prevention Study-II Nutrition Cohort, Patel et al. [3] observed a 31% reduced risk of aggressive PCa among men who performed >35 MET-h/wk of recreational physical activity compared with none. However, other studies have not shown associations with aggressive disease [6–8]. One factor potentially contributing to inconsistencies among studies is inadequate power to detect moderate associations with advanced and fatal PCa, which are less common. Another factor may be detection bias due to differential PSA testing by those engaged in high compared with low levels of physical activity. Our study and other studies [7,29] show that men who are more physically active are also more likely to get regular PSA testing, increasing subclinical disease detection. Although age- and multivariable-adjusted results were similar in our study, age-adjusted models showed a small positive association between vigorous activity and risk of localized PCa. Furthermore, we saw a reduction in risks of total, localized, and low-grade PCa among the highly screened subcohort. This exemplifies how this detection bias results in an apparent increased risk of PCa, potentially also masking a moderate inverse association.

PCa is characterized by substantial molecular heterogeneity [12]. Epidemiologic studies to evaluate specific molecular subtypes are, therefore, important to clarify PCa risk factors. Our results suggest that vigorous activity was associated with a reduced risk of *TMPRSS2:ERG*-positive but not *TMPRSS2:ERG*-negative PCa. This supports the hypothesis that presence of this fusion may define an etiologically distinct subtype of PCa. Our findings also support our hypothesis that physical activity may influence PCa risk through mechanisms related to *TMPRSS2:ERG*. One potential explanation is that physical activity influences whether or not a fusion event occurs. Cellular stress can contribute to genomic instability and is a trigger for genomic rearrangements [30]. It has been shown that physical activity may reduce oxidative stress and improve immune functions [31]. Thus, physical activity may protect against cellular stress, preventing the *TMPRSS2:ERG* fusion. Another possibility is that physical activity influences progression to clinical detection of tumors with the *TMPRSS2:ERG* fusion. We previously observed that expression of the insulin receptor and IGF-1 receptor was higher in *TMPRSS2:ERG*-positive cancers [18]. Regular physical activity may alter endogenous hormone levels, reducing availability of growth factors, such as insulin, and IGF-1 [9]. In this way, physical activity could influence tumor progression through these signaling pathways. Another explanation is that physical activity may lead to changes in prostate tumor vessel morphology that could influence the tumor's propensity to form metastases [32].

A limitation of this study is that ERG status was only known for a subset of PCa cases. To mitigate this potential issue, we applied inverse probability weighting to account for clinical factors, which yielded similar results as the unweighted analysis [20,21]. Given that our study is observational in design, there is potential for bias due to confounding by factors associated with both physical activity and risk of PCa. However, our analysis accounted for a wide range of health and lifestyle factors. One concern in epidemiologic studies of physical activity is reverse causation since men with preclinical disease may decrease their activity level prior to diagnosis. We examined cumulative average physical activity in our study, which is less likely to be influenced by preclinical disease. Strengths of our study include the integration of molecular tumor data with 26 yr of prospective cohort follow-up. This allowed us to examine the association between long-term physical activity and advanced, lethal, and *TMPRSS2:ERG*-postive and negative PCa. Since physical activity was prospectively collected using a validated questionnaire [23], measurement error is expected to be nondifferential with respect to incident PCa.

5. Conclusions

Our study found that vigorous physical activity over the long term is associated with a lower risk of clinically meaningful endpoints, including advanced, lethal, and *TMPRSS2:ERG*-positive PCa. These findings suggest that regularly engaging in higher levels of vigorous activity may be beneficial to men for prevention of clinically important PCa. Furthermore, these results suggest that physical activity may act through pathways related to development

of *TMPRSS2:ERG* and support the hypothesis that this subtype has unique etiological factors.

Author contributions: Claire H. Pernar had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Pernar, Mucci.
Acquisition of data: Mucci, Giunchi, Lis, Finn, Fiorentino.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: Pernar.
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Statistical analysis: Pernar, Ebot, Parmigiani.
Obtaining funding: Mucci.
Administrative, technical, or material support: None.
Supervision: Mucci.
Other: None.

Financial disclosures: Claire H. Pernar certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This work was supported by the National Institutes of Health (T32 ES 007069 to C.H.P., R25 CA 112355 to R.E.G., T32 CA 09001 to C.H.P. and S.C.M., P50 CA 090381 to L.A.M. and S.C.M., R01 CA 136578 to L.A.M., 4P30CA006516-51 to L.A.M. and G.P.); the Prostate Cancer Foundation Young Investigator Awards to L.A.M., K.M.W., K.H.S. and S.P.F.; the World Cancer Research Fund (2013/1003 to S.P.F. and L.A.M.). The Health Professionals Follow-up Study is supported by U01 CA 167552 from the National Cancer Institute. The TMAs were constructed by the Tissue Microarray Core Facility at the Dana-Farber/Harvard Cancer Center (P30 CA 06516).

Acknowledgments: We would like to thank the participants and staff of the HPFS for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. In particular, we would like to acknowledge Elizabeth Frost-Hawes, Siobhan Saint-Surin, Robert Sheahan, Ann Fisher, and Scott Smith.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.09.041.

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