Cancer Research

Exercise and Prognosis on the Basis of Clinicopathologic and Molecular Features in Early-Stage Breast Cancer: The LACE and Pathways Studies

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Abstract

To investigate whether the impact of postdiagnosis exercise on breast cancer outcomes in women diagnosed with early-stage breast cancer differs on the basis of tumor clinicopathologic and molecular features. Using a prospective design, 6,211 patients with early-stage breast cancer from two large population-based cohort studies were studied. Age-adjusted and multivariable Cox regression models were performed to determine the relationship between exercise exposure (total MET-hours/week) and recurrence and breast cancer–related death for: (i) all patients ("unselected" cohort), and on the basis of (ii) classic clinicopathologic features, (iii) clinical subtypes, (iv) PAM50-based molecular intrinsic subtypes, and (v) individual PAM50 target genes. After a median follow-up of 7.2 years, in the unselected cohort ($n = 6{,}211$) increasing exercise exposure was not associated with a reduction in the risk of recurrence (adjusted $P_{trend} = 0.60$) or breast cancer–related death (adjusted $P_{trend} = 0.39$). On the basis of clinicopathologic features, an exercise-associated reduction in breast cancer–related death was apparent for tumors <2 cm [HR, 0.50; 95% confidence interval (CI), 0.34–0.72], well/moderately differentiated tumors (HR, 0.63; 95% CI, 0.43–0.91), and ER-positive tumors (HR, 0.72; 95% CI, 0.53–0.97). Stratification by clinical subtype indicated that the $ER^+/PR^+/HER2^-$/low-grade clinical subtype was preferentially responsive to exercise (recurrence: adjusted HR, 0.63; 95% CI, 0.45–0.88; breast cancer–related death: adjusted HR, 0.57; 95% CI, 0.37–0.86). The impact of exercise on cancer outcomes appears to differ as a function of pathologic and molecular features in early-stage breast cancer. *Cancer Res; 76(18); 5415–22. ©2016 AACR.*

Introduction

In recent years a new line of investigation has emerged addressing the novel question of whether post-diagnosis exercise exposure affects cancer outcomes following a diagnosis of early-stage breast cancer. In a recent meta-analysis of seven observational studies ($n = 19{,}607$ patients), higher levels of post-diagnosis self-reported physical activity [≥15 metabolic equivalent tasks (MET)-hours/week] were associated with a significant 29% reduction in the risk of breast cancer–related death, compared with inactive patients (<5 MET-hours/week; ref. 1). Other meta-analyses and systematic reviews corroborate these findings (2–4), leading to the general conclusion that exercise performed after a diagnosis is associated with improvements in cancer outcomes.

The results of individual studies, however, are not consistent. Specifically, not all observational studies found that exercise was associated with a significant reduction in breast cancer-specific mortality (1). Furthermore, in those reporting a significant inverse relationship, the magnitude of exercise-associated risk reduction in breast cancer-specific mortality varied considerably (3). Finally, the "dose" of exercise found to confer observed risk reductions also varied considerably, ranging from ≥9 MET-hours/week (equivalent to walking briskly for 30 minutes, 5 days/week) to ≥21 MET-hours/week (brisk walking for 75 minutes, 5 d/wk; ref. 3).

Differences in study methodology related to the definition, timing, and most importantly, measurement of exercise exposure (i.e., reliability and validity of study instruments) undoubtedly contribute to the divergent findings. Other contributing factors include differences in study populations relating to demographic characteristics such as age and ethnicity but also medical characteristics, especially tumor subtypes. All observational studies investigating the exercise—breast cancer outcomes relationship have done so under the assumption that breast cancer is a single disease; however, human breast cancer displays considerable

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diversity in biology, therapeutic response, and overall outcomes (5). Although an increasing number of randomized trials demonstrate that exercise is associated with a benefit across a broad range of common physiological and psychosocial symptoms in women with early-stage breast cancer (6–8), the impact on tumor-related outcomes is less clear. Moreover, whether certain tumor subtypes are more responsive to the effects of exercise in early-stage breast cancer is largely unknown (9). Accordingly, we investigated the association between exercise exposure and cancer outcomes on the basis of tumor histologic and molecular features in women with early-stage breast cancer.

Patients and Methods

Patients and study overview

Women with histologically confirmed early breast cancer (AJCC stage I with tumor size $\geq$ 1 cm, stage II or stage IIIA) were selected from two population-based, prospective cohort studies, the Life After Cancer Epidemiology (LACE) study (10) and Pathways (11). In brief, major eligibility criteria for LACE were: (i) primary diagnosis of invasive breast cancer from 1996 to 2000, (ii) 18 to 79 years old at diagnosis, (iii) completion of primary adjuvant therapy, and (iv) no evidence of recurrence or progressive disease at study entry. Major eligibility criteria for Pathways included: (i) primary diagnosis of stage I-IV breast cancer from 2005 to 2008 and (ii) $\geq$21 years of age at diagnosis. Major exclusion criteria for both studies were bilateral disease and neoadjuvant therapy. LACE and Pathways protocols were reviewed and approved by the human subjects committee at Kaiser Permanente Northern California (KPNC; LACE and Pathways) and University of Utah (LACE only), and informed consent was obtained before study participation. A total of 6,641 breast cancer patients are enrolled in LACE and Pathways. Of these, exercise exposure data were available on 6,286; 75 patients with stage IV disease were also excluded for a final analytic cohort of 6,211 subjects.

Both cohorts were followed through April, 2015 and censored at the event date (recurrence or death), or date of last contact. Recurrence and breast cancer–related death were ascertained via self-report as well as computerized algorithm of KPNC electronic medical record review on a monthly basis among participants at least 6 months after initial primary diagnosis. All outcomes were verified by medical record review.

Breast cancer classification by immunohistochemical and PAM50 gene expression

Clinical breast cancer subtypes were defined using available clinical immunohistochemical (IHC) results for estrogen receptor (ER) and progesterone receptor (PR), and IHC and/or FISH for HER2, scored according to laboratory standards at the time of diagnosis for the entire cohort. We used a clinical subtype classification based on ER, PR, HER2 and histologic grade described previously (12) defined as follows: (i) ER^+, PR^+ and $HER2^-$ and well- or moderately differentiated (n = 1,900), (ii) ER^+, $PR^{+/-}$, $HER2^+$ and poorly or un-differentiated (n = 1,225), (iii) ER^-, ;PR^-, and $HER2^+$ (n = 159), and (iv) ER^-, PR^-, and $HER2^-$ (n = 446).

A stratified random sample of LACE and Pathways participants were selected for intrinsic subtyping. ER, PR, and HER2 status based on IHC (and/or FISH for HER2) defined the strata used for sampling, with an 18% random sample selected among cases of the common breast cancer phenotype that is positive for ER or PR expression and negative for HER2 and a 100% sample of tumors that were ER^- and PR^- or $HER2^+$ (13). The final analytic cohort size for the PAM50 analyses was 1,501 (12). Paraffin-embedded tissue blocks were collected from hospitals where patients underwent surgical resection of the primary tumor. All tissue sections were reviewed by one pathologist. Tissue punches were deparaffinized and digested for RNA extraction as described previously (14). Reverse transcriptase PCR (RT-qPCR) was conducted for the 50 target genes (i.e., PAM50) and 5 control genes (12, 15). Molecular intrinsic subtypes from PAM50 were determined using centroids from an independent RT-qPCR training set as previously described (12). Using this system, tumors were classified into the following groups: (i) luminal A (n = 544), (ii) luminal B (n = 333), (iii) HER2-enriched (HER2-E; n = 331), (iv) basal-like (n = 293), and (v) normal-like (n = 52). Tumors classified as normal-like were excluded from analyses. In addition, we also explored the relationship between exercise and recurrence for all genes comprising the PAM50 gene-expression signature.

Exercise exposure assessment

At the time of enrollment, participants in both cohorts completed a self-administered survey that assessed exercise behavior. Exercise exposure was assessed using items from the Arizona Activity Frequency Questionnaire (16) that evaluated different domains of physical activity, including leisure-time recreational activity (i.e., exercise). Non-recreational activity (e.g., occupational activity, activities of daily living) was not included in the calculation of exercise exposure. The validity and reliability of this instrument has been previously described (16). In brief, patients reported the frequency and duration of activities (e.g., walking, jogging, running, bicycling, swimming laps, racket sports) performed at least once a month in the past 6 (Pathways) or 12 months (LACE). Standardized MET values (17) were assigned to each activity, with "dose" being calculated by multiplying the frequency of activity sessions per week by average session duration, weighted by the standardized MET for the particular exercise modality. Individual activities were summed to derive a total MET-hours/week (MET-h/wk). Total MET-h/wk was categorized via an unbiased quartile split (<2, 2 to 10, >10 to 25, and >25 MET-h/wk).

Statistical analysis

Demographic, disease, and treatment characteristics are reported by quartiles of exercise exposure, and compared using χ^2 tests for categorical outcomes and ANOVA for continuous variables. We used the Q statistic to test for heterogeneity in the exercise—outcome risk estimates between the two independent cohorts. The Q statistic P levels for recurrence and breast cancer–related death were not significant supporting combining of cohorts into a pooled analysis. Cox proportional hazards regression models were used to estimate the HRs and 95% confidence intervals (CI) for the association between exercise exposure and recurrence or death from breast cancer, adjusted for covariates. Time since diagnosis was the time scale used in the regression models, allowing for delayed entry into the cohort (i.e., left truncation, with study entry ranging from 0 to 3.2 years post-diagnosis). Covariates were ascertained at the time of diagnosis via questionnaire, medical chart review, and tumor registry and were as follows: age, race, menopausal status, smoking, body mass index, tumor stage, adjuvant therapy (chemotherapy, radiation, endocrine, HER2-directed therapy), and comorbidity score (Charlson comorbidity index; ref. 18).

Regression estimates for PAM50 analyses accounted for the case-cohort study design with stratified breast cancer sub-cohort sampling (19), as implemented in SAS subroutines (20). Exploratory, hypothesis-generating Cox proportional hazards regression models were used to estimate the multivariable-adjusted HR and 95% CI for the association between exercise and cancer outcomes on the basis of standard clinicopathologic parameters (i.e., tumor size, tumor grade, ER status, and HER2 status) or clinical subtypes- or gene expression-based (i.e., PAM50) intrinsic subtypes, as well as the individual PAM50 target genes. Tests of interactions between exercise and recurrence were assessed by entering the cross product of dichotomized exercise exposure and the dichotomized covariate. Because of small sample size, all PAM50-related analyses were age-adjusted only. Statistical analyses were conducted using SAS Version 9.3 (SAS Institute, Inc.) and R Version 2.14.2 and Stata Version 10.1 (StataCorp). A significance probability less than 0.05 was considered statistically significant for all tests. Because of the exploratory nature, a significance level less than 0.20 was considered of interest for tests of interactions between exercise and PAM50 target genes with respect to recurrence. All statistical inferences were two-sided.

Results

LACE and Pathways participants were similar in age, family history of breast cancer, smoking history, and adjuvant therapy, as previously described (12). Patient characteristics are presented in Table 1. Median follow-up was 7.2 years. During this period, a total of 678 recurrences and 405 breast cancer–related deaths were observed.

Table 1. Demographic and treatment characteristics of the participants[a]

		MET·h/wk				
Characteristic	**Overall**	**<2**	**2–10**	**>10–25**	**>25**	***P***
No. of participants (%)	6,211 (100)	1,554 (25.0)	1,538 (24.8)	1,566 (25.2)	1,553 (25.0)	
Age (y) at diagnosis, mean (SD)	59.1 (11.7)	61.4 (11.5)	58.6 (11.9)	59.6 (11.6)	56.7 (11.2)	<0.0001
Time (mo) from diagnosis to enrollment, mean (SD)						
LACE	22.4 (6.7)	22.7 (6.7)	22.0 (6.6)	22.2 (6.5)	22.7 (7.1)	0.94
Pathways	2.1 (0.8)	2.0 (0.7)	2.1 (0.7)	2.0 (0.8)	2.1 (0.8)	0.71
Race (%)						<0.0001
Non-Hispanic white	69.5	67.8	65.7	72.8	71.6	
Other group	30.5	32.2	34.3	27.2	28.4	
BMI (kg/m^2), mean (SD)	27.9 (6.4)	30.1 (7.4)	28.3 (6.5)	27.1 (5.5)	26.0 (5.3)	<0.0001
Smoking (%)						<0.0001
Never	55.6	51.6	56.6	55.0	59.1	
Former	38.9	38.7	37.8	41.2	37.8	
Current	5.6	9.7	5.6	3.8	3.2	
Menopausal status (%)						<0.0001
Postmenopausal	69.1	75.0	69.1	70.1	62.1	
Premenopausal	27.0	21.4	26.4	25.9	34.3	
Undetermined	4.0	3.6	4.5	4.1	3.7	
AJCC stage (%)						0.02
I	52.8	50.9	52.1	51.7	56.5	
II	39.4	40.9	39.2	40.5	37.1	
III	7.8	8.2	8.6	7.9	6.4	
Tumor size, cm (%)						0.10
<2 cm	60.3	58.5	59.2	61.1	62.5	
≥2 cm	39.7	41.5	40.8	38.9	37.5	
Tumor grade (%)						0.55
Well or moderately differentiated	71.3	72.6	70.4	70.7	71.5	
Poor/undifferentiated	28.7	27.4	29.6	29.3	28.5	
ER positive (%)	83.1	83.5	82.3	81.6	84.9	0.08
PR positive (%)	65.5	65.6	65.9	65.3	65.0	0.97
HER2 positive	14.3	13.5	15.2	14.3	14.0	0.64
Clinical subtype[b] (%)						0.04
ER$^+$/PR$^+$/HER2$^-$/low-grade	50.8	51.4	50.5	51.2	49.9	
ER$^+$/PR$^{-/+}$/HER2$^+$	33.0	32.5	32.3	31.2	35.9	
ER$^-$/PR$^-$/HER2$^+$	4.5	4.0	5.7	4.6	3.6	
ER$^-$/PR$^-$/HER2$^-$	11.8	12.1	11.5	13.0	10.6	
Treatment characteristics (%)						
Surgery type						0.45
Lumpectomy	57.4	58.0	56.2	56.2	59.0	
Mastectomy	42.3	41.5	43.6	43.4	40.7	
None	0.3	0.5	0.2	0.4	0.3	
Received chemotherapy	49.5	45.8	51.0	50.9	50.1	0.01
Received radiotherapy	49.7	48.5	49.8	51.0	49.5	0.59
Received tamoxifen or AI	70.9	71.1	71.6	69.6	71.4	
Charlson comorbidity score (%)						<0.0001
None	86.3	80.4	86.5	87.2	91.4	
≥1	13.7	19.6	13.5	12.8	8.6	

Abbreviations: AI, aromatase inhibitor; AJCC, American Joint Committee on Cancer; BMI, body mass index.

[a]Percentages are weighted because of stratified case–cohort study design.

[b]Clinical subtypes based on 3-marker IHC plus grade, adapted from St. Gallen's Consensus Conference.

Table 2. Age-adjusted and multivariable-adjusted hazard ratios according to quartile of exercise behavior (MET-hrs.wk^{-1}) for 'unselected' cohort

		MET.hrs.wk^{-1}				
	Total (n = 6,211)	**<2 (n = 1,554)**	**2 to 10 (n = 1,538)**	**>10 to 25 (n = 1,566)**	**>25 (n = 1,533)**	**P_{trend}**
Recurrence—No. of events	678	180	176	167	155	
Median time to recurrence, years (range)		3.8 (0.5–15.5)	3.6 (0.5–15.9)	3.8 (0.3–16.0)	3.8 (0.6–17.0)	
Age-adjusted HR (95% CI)		1.00	0.97 (0.78–1.19)	0.90 (0.73–1.11)	0.85 (0.68–1.06)	0.05
Multivariable-adjusted HR (95% CI)[a]		1.00	1.00 (0.81–1.24)	0.92 (0.74–1.15)	1.01 (0.80–1.27)	0.60
Breast cancer deaths—No. of events	405	118	102	96	89	
Median survival, years (range)		5.5 (0.7–15.7)	4.9 (0.7–16.5)	6.0 (0.3–16.5)	5.3 (1.0–14.4)	
Age-adjusted HR (95% CI)		1.00	0.87 (0.66–1.13)	0.79 (0.60–1.04)	0.79 (0.59–1.05)	0.02
Multivariable-adjusted HR (95% CI)[a]		1.00	0.90 (0.68–1.19)	0.82 (0.62–1.10)	1.00 (0.74–1.34)	0.39

Abbreviations: MET, metabolic equivalent task; HR, hazard ratio; CI, confidence interval.

[a]adjusted for age at diagnosis (<50 yrs vs. $\geq$ 50); race, smoking status; body mass index; menopausal status; tumor stage; chemotherapy treatment (yes vs. no); radiation therapy (yes vs. no); trastuzumab (yes vs. no); hormone therapy (yes vs. no); comorbidity score (any vs. none).

"Unselected" cohort analysis

Table 2 presents the age-adjusted and the multivariable-adjusted models for recurrence and breast cancer–related death according to total MET-h/wk quartiles. In age-adjusted analysis, exercise exposure above the referent group (<2 MET-h/wk) was associated with a significant reduction in recurrence (P_{trend} = 0.05; Table 2; Fig. 1A) and breast cancer–related death (P_{trend} = 0.02; Table 2; Fig. 1B). Both linear trends became non-significant in the multivariable model (recurrence: P_{trend} = 0.60; breast cancer–related death: P_{trend} = 0.39; Table 2).

Given limited evidence for a dose–response relationship, a smoothing spline was generated of the log hazard (for breast cancer–related death) versus the log of total MET-h/wk to identify the optimal exercise exposure. The spline indicated the most robust response to exercise was apparent for exposure above zero up to approximately 10 MET-h/wk (Supplementary Fig. S1). On this basis, exercise was collapsed into two categories [no exercise behavior (i.e., 0 MET-h/wk; n = 852) vs. >0 MET-h/wk (n = 5,359)] for all subsequent analyses. Compared with 0 MET-h/wk, the adjusted HR for recurrence and breast cancer–related death was 0.89 (95% CI, 0.71–1.10) and 0.74 (95% CI, 0.57–0.97) for >0 MET-h/wk, respectively.

Clinicopathologic features analysis

Compared with 0 MET-h/wk, exercise was not associated with a reduction in recurrence on the basis of any clinicopathologic features (Fig. 2A). For breast cancer–related death, the impact of

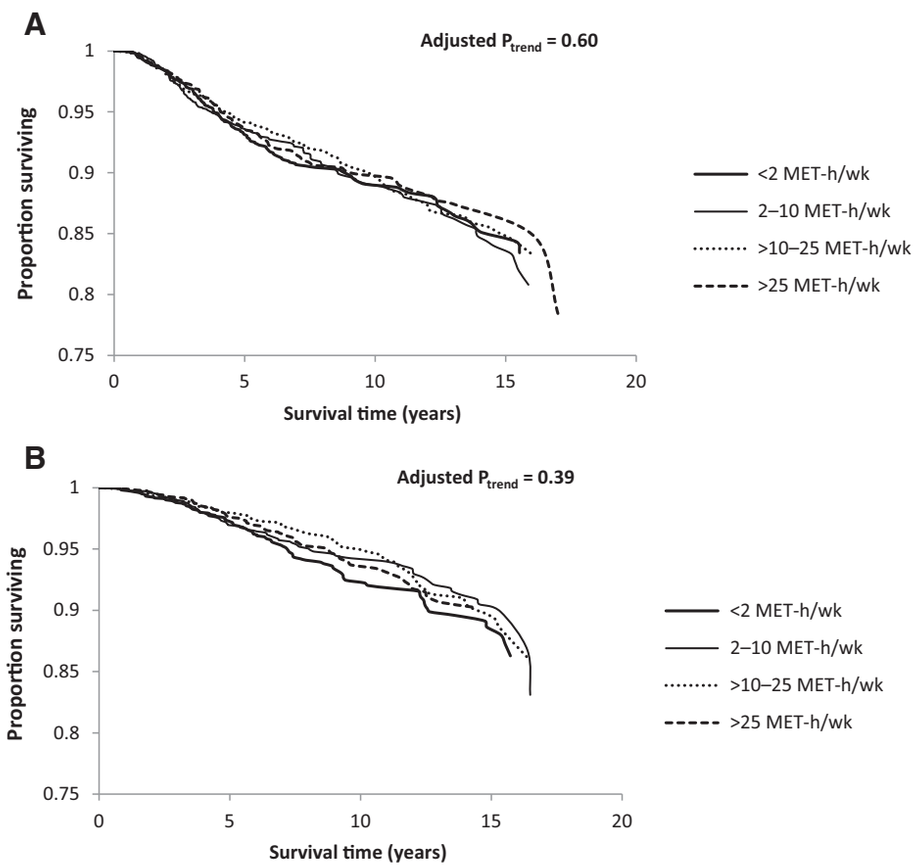

Figure 1.
Kaplan–Meier plots for recurrence (**A**) and breast cancer–related death (**B**) according to quartile of exercise category (MET-h/wk).

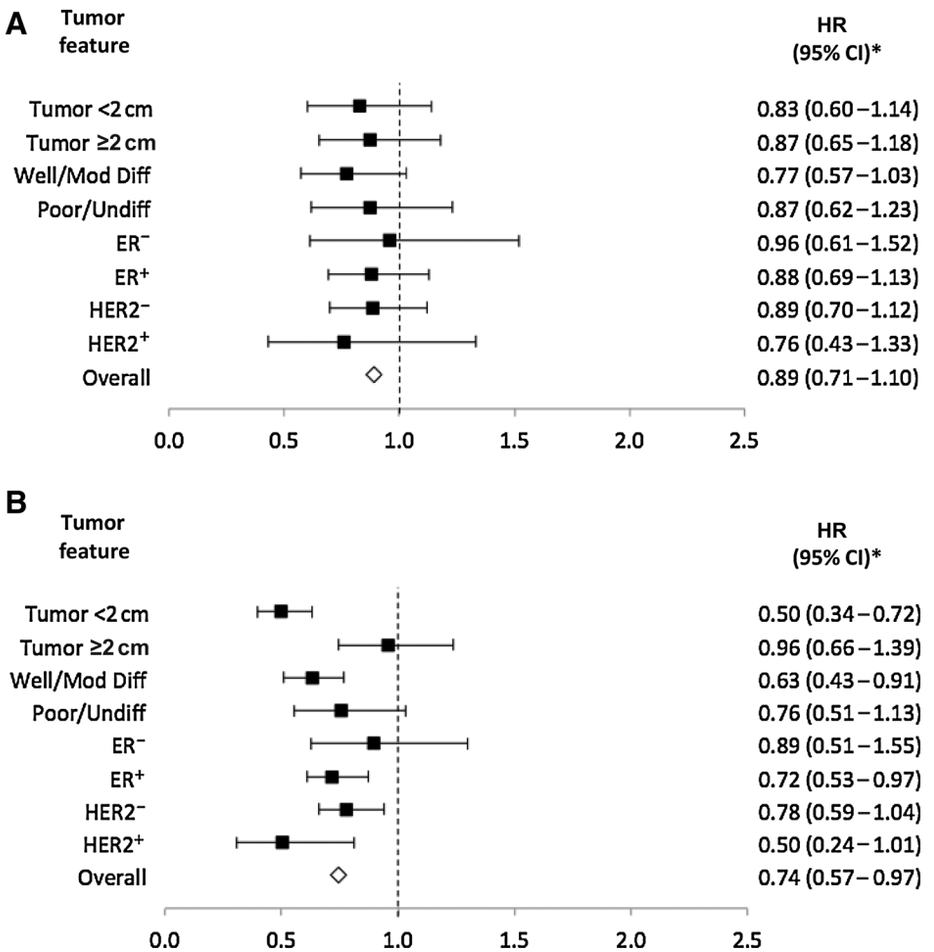

Figure 2.
Forest plots for recurrence (**A**) and breast cancer–related death (**B**) according to dichotomized exercise exposure (0 MET-h/wk vs. >0 MET-h/wk) on the basis of standard clinicopathologic features. Well/mod diff, well/moderately differentiated; poor/undiff, poorly differentiated; *, multivariable adjusted analyses.

exercise differed as a function of tumor size ($P_{interaction} = 0.02$), with a reduction only apparent in tumors <2 cm (HR, 0.50; 95% CI, 0.34–0.72) and not tumors ≥2 cm (HR, 0.96; 95% CI, 0.66–1.39; Fig. 2B). There were no significant interactions between exercise and breast cancer–related death on the basis of grade, ER, or HER2 status ($P > 0.05$), although the risk of death was significantly lower for exercising patients with well/moderately differentiated tumors (HR, 0.63; 95% CI, 0.43–0.93) and ER positive (HR, 0.72; 95% CI, 0.53–0.97) tumors; there was a suggestion that an exercise-associated reduction in breast cancer–related death was apparent for HER-2–positive tumors (HR, 0.50; 95% CI, 0.24–1.01).

Clinical or molecular intrinsic subtypes, and PAM50 target gene analysis

Clinical subtypes. An exercise-associated reduction in recurrence and breast cancer–related death was only apparent in the $ER^+/PR^+/HER2^-$/low-grade clinical subtype (HR, 0.63; 95% CI, 0.45–0.88 and HR, 0.57; 95% CI, 0.37–0.86, respectively; Fig. 3A), although the tests for interactions were not significant (recurrence: $P_{interaction} = 0.11$; breast cancer–related death, $P_{interaction} = 0.09$).

Molecular intrinsic subtypes. In comparison with 0 MET-h/wk, exercise >0 MET-h/wk was not associated with a reduction in recurrence or breast cancer–related death on the basis of any intrinsic subtype. The magnitude of risk reduction for luminal A tumors was not statistically significant yet was similar to that for $ER^+/PR^+/HER2-$tumors (recurrence: HR, 0.74; 95% CI, 0.38–1.43; breast cancer–related death: HR, 0.55; 95% CI, 0.27–1.12; Fig. 3B).

PAM50 target gene analysis. Exploratory analysis of the individual PAM50 target genes revealed a potential exercise and recurrence interaction ($P < 0.20$) for six genes (*ANLN*, *CCNE1*, *CDC6*, *EXO1*, *KIF2C*, and *KNTC2*). All genes, with the exception of *TMEM45B*, have critical roles in cell proliferation; on this basis, we calculated a 6-metagene signature. Using a median split on metagene expression level, low proliferation index tumors (HR, 0.58; 95% CI, 0.34–0.99) appeared to be more responsive to exercise as compared with high proliferation index tumors (HR, 0.84; 95% CI, 0.45–1.58), although the test for interaction was only marginally significant ($P_{interaction} = 0.08$).

Discussion

In this large, prospective cohort study we found that increasing exercise exposure was not associated with a reduction in the risk of recurrence or breast cancer–related death in an "unselected" cohort of women with early-stage breast cancer. However,

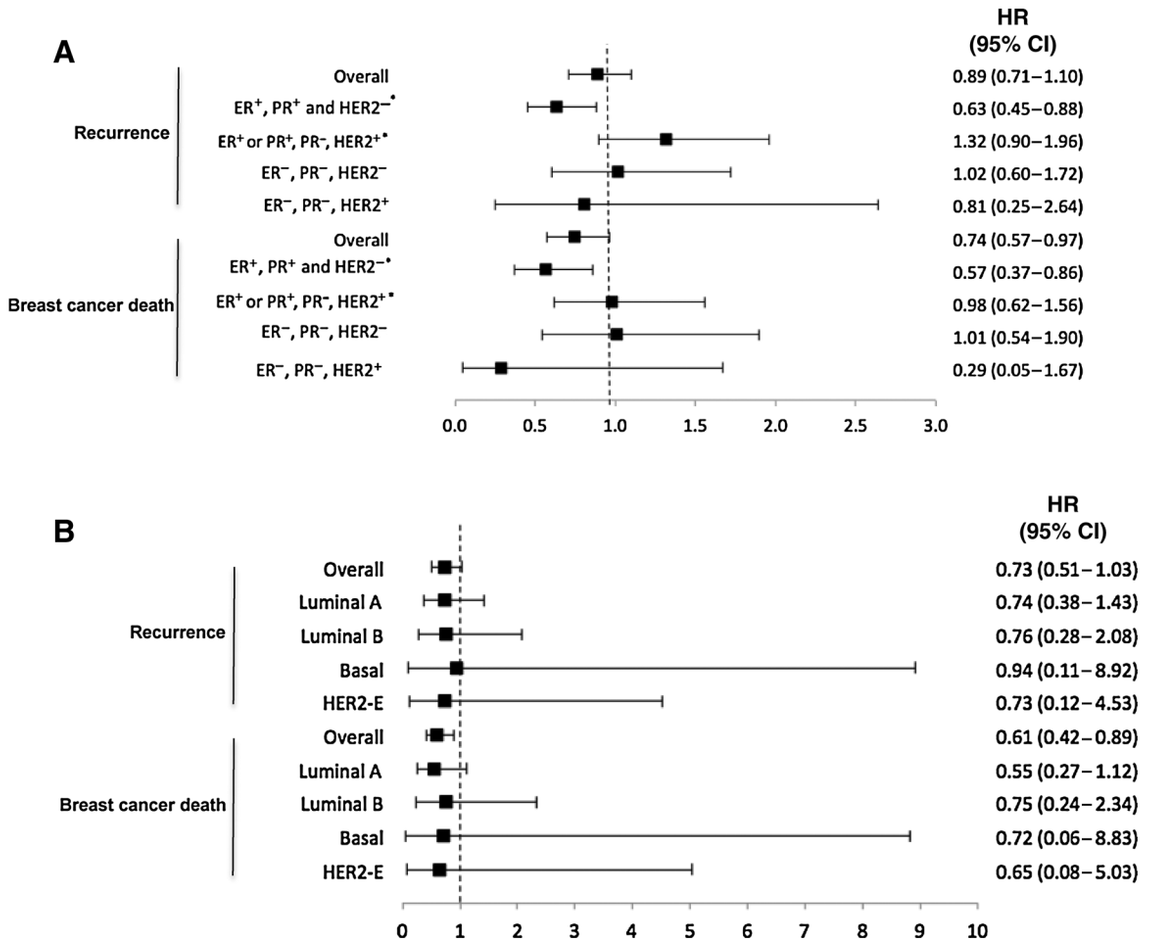

Figure 3.

Forest plots for recurrence and breast cancer–related death according to dichotomized exercise exposure (0 MET-h/wk vs. >0 MET-h/wk) on the basis of clinical subtypes (*, multivariable adjusted analyses; **A**) and PAM50-based molecular intrinsic subtypes (*, age-adjusted; **B**). LumA, luminal A; LumB, luminal B; NNN, triple negative; HER2, HER2-enriched; HER2-E, HER2-enriched.

considerable biological diversity exists across breast cancer subtypes that, in turn, have led to the development and approval of subtype-specific treatment strategies (21). Hence, within such diversity, the possibility that a specific subpopulation(s) may preferentially derive benefit from exercise treatment is biologically plausible. Here, we sought to address this question under the assumption that breast cancer represents multiple diseases (as opposed to a single disease), and determined whether the impact of exercise on breast cancer–specific outcomes differed on the basis of these subpopulations. Overall, our data indicate that the benefit of exercise on breast cancer–specific outcomes differs on the basis of tumor characteristics. It is important to state that the present results do not, of course, negate the demonstrated benefit of exercise on other non-tumor related health outcomes (e.g., various patient-reported and physiological outcomes) in early-stage breast cancer. Nevertheless, they do however create a strong rationale that the investigation of exercise treatment as a candidate antitumor strategy needs to adopt a precision/translational oncology approach wherein exercise is matched to the patient on the basis of tumor characteristics, and also possibly genotype (22).

To our knowledge, this is the first report indicating that smaller tumor size and lower grade tumors may be more responsive to exercise treatment. Although the mechanistic underpinnings are not known, we speculate that tumor cell biology (e.g., differences in proliferation rates) and/or effect of prescribed treatments (e.g., differences in adjuvant therapy, endocrine therapy ± chemotherapy, for small vs. large or low vs. high-risk tumors) factors may be partially responsible. Secondary analyses from a limited number of prior reports corroborate our finding that hormone receptor–positive tumors may be preferentially responsive to exercise. For example, in a retrospective analysis of 2,987 early breast cancer patients, Holmes and colleagues (23) found that exercise exposure (≥9 MET-h/wk) was associated with a significant 50% reduction in breast cancer–related death in ER positive tumors compared with a non-significant 9% reduction in ER-negative tumors. Our novel finding that the clinical subtype of $ER^+/PR^+/HER2^-$/low-grade by IHC appear particularly responsive to exercise, in combination with the lack of benefit in hormone receptor–negative tumors, further supports the notion that hormone receptor status may be an important

feature modulating the exercise—breast cancer outcomes relationship.

Breast cancer progression is regulated by complex, multifaceted interactions between the systemic milieu (host), tumor microenvironment, and tumor histologic/molecular features (24). Thus, efficacy of exercise treatment to improve breast cancer outcomes is likely dependent on modulation of the host–tumor interaction (25), and/or augmentation of anticancer therapy efficacy (26). The enhanced responsiveness of ER positive and luminal A tumors is worth considering within this paradigm. First, The Cancer Genome Atlas analysis indicated critical differences in the major tumor suppressors, with ER-positive/luminal A tumors retaining activity of retinoblastoma/*RB1* and *TP53* compared with a high frequency of *RB1* and *TP53* (as well as *PTEN*) mutations in ER-negative tumors (5). It is plausible that such pathways may play a critical role in mediating the response to exercise treatment. Indeed, preclinical work showed that exercise treatment was associated with inferior survival and augmented tumorigenesis compared with sedentary control in the *p53*-deficient MMTV-Wnt-1 transgenic mouse model of breast cancer (27). Second, the majority of small luminal A tumors are classically treated with endocrine therapy that blocks estrogen action. Enhanced sensitivity of these tumors raises the intriguing possibility that exercise could augment the antitumor efficacy of adjuvant endocrine therapy such that action is more complete. Interestingly, chronic aerobic training causes minimal reductions in circulating sex steroid hormone concentrations in postmenopausal healthy women (28) although exercise could, of course, modulate ER action via non-classical pathways (29).

Despite data showing the preferential benefit of ER-positive tumors to exercise (23, 30), our clinical subtypes and novel PAM50 findings indicate that stratification of tumors into more biologically homogenous diseases may identify those that are more responsive to exercise treatment, as well as those that may not derive benefit. Further interrogation of PAM50 target genes indicated that low-proliferative index tumors might be responsive to exercise. The mechanistic basis for this finding is not known; however, this finding should be interpreted with caution due to the overlapping confidence intervals, the lack of sufficient power, and required validation in independent cohorts. Hence, in addition, our present findings also do not rule out the possibility that a subset of aggressive tumors may also benefit from exercise treatment. Indeed, the considerable variability within pathologic/molecular classification systems adopted in the present study suggests that other unidentified tumor cell-intrinsic features as well as features of the numerous cell types in the tumor microenvironment likely further modify exercise response. To this end, using IHC-based molecular target analysis, several specific tumor biomarkers have been shown to predict exercise response in early-stage colorectal cancer, with *PTGS2*-positive, *CTNNB1*-negative, and *CDKN1B* (p27)–expressing tumors appearing preferentially responsive to exercise (31–33). High-throughput genomic and transcriptomic expression profiling platforms (34) will prove powerful approaches to untangling the highly complex underpinnings of the exercise - tumor response, as well as provide much needed insight into mechanisms of action.

Self-report measures of exercise behavior have well-known limitations, and therefore some misclassification of exercise exposure is expected. Relatedly, it is not possible to delineate whether exercise participation simply reflects lower disease and/or treatment-related toxicities as opposed to direct exercise-induced effects. Analyzing the impact of exercise in the context of clinical trials is preferable wherein exercise exposure can be accurately verified; however, the use of well-designed molecular epidemiologic studies is an essential first step when adopting a precision oncology approach to the investigation of exercise as a candidate anticancer treatment. Finally, exercise exposure was assessed at only one point following diagnosis but also at different time points between the two combined study cohorts (i.e., past 6 months in Pathways and past 12 months in LACE). Timing of exercise exposure assessment after diagnosis may affect the relationship between exercise and tumor outcomes especially if assessed during versus after the completion of primary adjuvant therapy. It will be important for future studies to elucidate the impact of post-diagnosis exercise exposure that is performed during versus after adjuvant therapy as well as change in exercise on outcomes.

In conclusion, the impact of exercise on cancer outcomes appears to differ across tumor subpopulations in early-stage breast cancer. Our findings have important implications for future work investigating the efficacy of exercise treatment as a candidate anticancer strategy in breast and other solid tumors.

Disclosure of Potential Conflicts of Interest

P.S. Bernard has ownership interest (including patents) in Bioclassifier LLC. C.T. Dang reports receiving a commercial research grant from Roche/Genentech and GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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