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Association of prediagnostic physical activity with survival following breast cancer diagnosis: influence of *TP53* mutation status

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Abstract

Purpose—Physical activity both before and after breast cancer diagnosis has been associated with improved survival. However, it is not clear whether this association differs by molecular features of the tumor or by recency of the physical activity to the time of diagnosis.

Methods—We examined the association of prediagnostic physical activity with survival in a cohort of 1,170 women with primary, incident, and histologically confirmed breast cancer, examining tumor molecular subtypes. Cox regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI).

Results—Mean follow-up time was 87.4 months after breast cancer diagnosis; there were 170 deaths identified. Compared with inactive patients (<3 hours/ week), women with higher average lifetime physical activity (>6 hours/ week) had reduced risk of all-cause mortality (adjusted HR = 0.61, 95% CI, 0.40-0.95; *p* trend = 0.04). There were no clear differences in the associations for lifetime and more recent physical activity. Lifetime physical activity was also weakly associated with decreased risk of breast cancer-specific mortality. Higher lifetime physical activity was associated with reduced risk of all-cause mortality among women with ER-positive tumors (HR =

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0.52, 95% CI, 0.29-0.93), and mutant *TP53* tumors (HR = 0.22, 95% CI, 0.06-0.72); however, no statistically significant interactions were observed for ER or *TP53* status.

Conclusions—Our study further supports that prediagnostic physical activity improves overall survival following breast cancer, and suggests that the associations of prediagnostic physical activity with survival following breast cancer may vary by molecular features of the tumor, particularly ER and *TP53* status.

Keywords

physical activity; breast cancer survival; *TP53* mutation; epidemiology

Introduction

There is consistent evidence that pre- and post-diagnostic physical activity is associated with improved survival of breast cancer [1-5]. While many studies have examined prognosis following breast cancer as a whole, molecular subtypes of breast cancer associated with specific clinical characteristics may differ in prognosis. Women with estrogen receptor (ER) and progesterone receptor (PR) positive breast cancer have better survival than those with hormone receptor negative tumors [6, 7], while patients with *HER2*⁺ tumors have higher rates of recurrence and breast cancer mortality than those with *HER2*⁻ tumors [8]. However, only a few studies have examined the association between prediagnostic physical activity and breast cancer survival stratified by hormone receptor status [9-11]; results have been inconsistent.

Mutation in *TP53* is another important and independent molecular marker of poor prognosis for breast cancer [12-14]. The p53 protein plays multiple roles in regulation of cell cycle, DNA repair, apoptosis, cellular differentiation, and senescence [15]; p53 also can down-regulate glucose metabolism, enhance mitochondrial respiration and ATP generation by increasing the conversion of glutamine into glutamate, as well as facilitate oxidative phosphorylation by promoting the assembly of Complex I and IV of the respiratory chain [16-20]. These findings suggest that *TP53* mutations contribute to alterations in the cell metabolism towards increased glycolysis and decreased energy production through mitochondrial respiration. These conditions are characteristic of aerobic glycolysis, the so-called “Warburg effect”, a frequent characteristic of tumor cells [21, 22]. Physical activity, especially moderate intensity aerobic physical activity, is known to enhance mitochondrial and oxidative capacities by promoting the activity of enzymes involved in glucose and fatty acid oxidation [23, 24]. Previous studies have reported that over-weight or reduced physical activity is associated with p53 overexpression, a common feature of missense *TP53* mutation, in colorectal cancer [25]. However, to our knowledge, no previous studies have examined the association of physical activity with survival following breast cancer examining the *TP53* mutation status of the breast tumors.

In a population-based study of breast cancer in western New York State, we investigated associations of pre-diagnostic physical activity and survival, examining breast cancer molecular subtypes.

Materials and Methods

Study population

Detailed study methods have been published previously [26-28]. Briefly, a population-based case-control study, the Western New York Exposures and Breast Cancer (WEB) Study, included 1,170 women aged 35-79 years with incident, primary, histologically confirmed

breast cancer. Cases were interviewed within one year of diagnosis; most (64%) were interviewed within 3-6 months following the diagnosis. All participants provided informed consent, and the study protocol was approved by the Institutional Review Boards of the University at Buffalo and all participating institutions.

Extensive interviewer-administrated and self-administered questionnaires were completed by participants, including queries regarding demographic factors, medical history, menstrual and reproductive history, tobacco and alcohol use, and other breast cancer risk factors. Information on lifetime physical activity was collected. Participants were asked to indicate how often and how many hours per week they regularly did any sports, strenuous leisure time physical activity or exercise, vigorous household chores, or manual yard work for time periods up to a year before the breast cancer diagnosis. Physical activity was queried for the following time periods: ages 10-13, 14-18, 19-22, 23-34, 35-50, 51-64, and over 65. From these reports, we calculated average physical activity over the lifetime (expressed as hours per week of physical activity from age 10 to one year before diagnosis), and for the 1-10 years and 10-20 years prior to diagnosis. In addition, because the 2008 Physical Activity Guidelines recommend engaging in at least 2.5 hours of moderate intensity physical activity per week to improve health [29], participants who averaged <3 hours per week were classified as low activity, those spending 3-6 hours per week as moderate, and those spending >6 hours per week as high activity. We further calculated percentage of time being more active (>3 hours per week) over the lifetime for participants. BMI was calculated as body weight in kilograms divided by the square of height in meters (weight (kg)/ height (m)²).

Information on tumor size, histological grade, cancer stage (as measured by tumor-node-metastasis (TNM) stage), and cancer treatment was abstracted from medical records by trained research nurses using a standardized protocol. The pathological diagnosis of breast cancer was reconfirmed by a senior pathologist from Georgetown University.

Tumor block determinations

Archived tumor blocks were successfully obtained from 920 (78.6%) of all participant breast cancer cases. ER and PR status were determined in tumor blocks at the Lombardi Cancer Center at Georgetown University by a single pathologist using immunohistochemical (IHC) analysis as described previously [28]. For patients for whom tumor blocks were unavailable or for whom hormone receptor status was unable to be determined (e.g., insufficient tumor tissue), hormone receptor status was obtained from hospital chart review. Using both methods, ER status was determined for 91% (752 ER-positive and 315 ER-negative) and PR status for 90% (656 PR-positive and 392 PR-negative) of cases. HER2 protein expression was also determined by the same pathologist using IHC for each sample. HER2 was scored using the guidelines of HerceptTest™ as described previously [28]. For patients for whom HER2 expression could not be determined, HER2 status data were obtained from patients' hospital charts. HER2 expression data were available from one method or the other for 64% (n = 74 and 677 for HER2- positive and negative, respectively) of cases. For patients for whom we had data regarding ER, PR or HER2 status from both laboratory assessment and from their medical record, agreement between sources was good (ER: $\kappa = 0.66$, PR: $\kappa = 0.73$, HER2: $\kappa = 0.65$) [28].

TP53 mutation status was determined using Affymetrix p53 Gene Chip System (Affymetrix, Santa Clara, CA) as previously described [30]. Briefly, this assay detected base substitutions, short deletions and insertions in exons 2 to 11 of *TP53* (encompassing the entire coding region and flanking splice junctions), followed by hybridization of the PCR product on the array. The presence of mutation was subsequently confirmed by bidirectional sequencing using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems,

Foster City, CA) and the Mega BACE 100 DNA Analysis System (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) according to the manufactures' instructions. Data were available for 63% of cases; 28% (205) were found to have a *TP53* mutation and 72% (528) were *TP53* wild-type.

Vital status through the end of 2006 was determined through matching of participant records with the National Death Index data. Survival time was calculated as the time from cancer diagnosis to the study endpoints, censoring at the date of December 31, 2006 or date of death. All-cause mortality was defined as any death, and underlying causes of death were broadly classified as breast cancer, other cancer, cardiovascular diseases, and all others.

Statistical analysis

The Cox proportional hazards regression model was used to evaluate the association between prediagnostic physical activity and the risk of all-cause and breast cancer-specific mortality. We considered as potential confounders in multivariable modeling known and suspected prognostic factors of breast cancer including age at diagnosis, race, BMI, education, menopausal status, stage of breast cancer at diagnosis, and status of cancer treatment, such as radiotherapy, chemotherapy, and hormonal therapy, as well as tumor characteristics including ER, PR and HER2 status and *TP53* mutation. Characteristics that altered the point estimates by 10% or more were included in the final model. BMI was classified into three categories: <25.0, 25.0-29.9, and ≥30.0, categories of normal, overweight, and obese, respectively. Because of evidence that there may be differences in the causal pathway for tumors with different clinical characteristics, we further evaluated the associations of prediagnostic physical activity with the study outcomes stratifying on menopausal status, ER, PR, HER2 status, and *TP53* mutation status, and examined potential interactions between prediagnostic physical activity and these molecular markers by evaluation of a multiplicative term in the Cox regression model. We also used the fractional polynomials method to assess trends for the continuous physical activity variable in the multivariable Cox model, no nonlinearity was detected [31]. Because silent mutations may not alter p53 function, we also evaluated effect modification by *TP53* status among patients with non-silent mutations and found similar results. Those results are not shown. All statistical tests were based on two-sided probability. Statistical analyses were conducting using SAS, Version 9.2 (SAS Institute, Cary, NC).

Results

During the study period, 170 of the 1,170 patients died. Of those deaths, 100 were from breast cancer. Mean follow-up time was 87.4 months (standard deviation (SD): 20.8, range: 9.0-125.0 months). Table 1 summarizes selected patient characteristics at time of questionnaire completion. Compared to women alive through 2006, those who died were slightly older and less likely to be Caucasian, less educated, had a higher BMI, higher TNM stage, and more likely to have radiotherapy, and/or chemo treatment for the breast cancer, and to have a tumor that was ER⁻, PR⁻, or with a *TP53* mutation. The distribution and types of *TP53* mutations we detected in this series was comparable to the spectrum of published breast cancer mutations compiled in the International Agency for Research on Cancer (IARC) published database (<http://p53.iarc.fr/>).

Associations of prediagnostic physical activity with all-cause and breast cancer-specific mortality after adjustment for age at diagnosis and other known prognostic factors for breast cancer are presented in Table 2. Patients with highest average lifetime physical activity (>6 hours/ week) had lower risk of all-cause mortality than those in the lowest level (HR = 0.61, 95% CI, 0.40-0.95; *p* trend = 0.04). When considering the percentage of their lifetime that a participant was active more than 3 hours/week, the adjusted HR for all-cause mortality

associated with 75% of lifetime compared to <25% of lifetime was 0.60 (95% CI, 0.39-0.95; p trend = 0.02). Higher level of physical activity 1-10 years before diagnosis was associated with reduced, though not significantly reduced risk of death from any cause (HR = 0.73, 95% CI, 0.50-1.08 for physical activity >6 compared to <3 hours/ week). A similar inverse association was found between high level of physical activity 10-20 years prior to diagnosis and all-cause mortality. Additionally, we examined the association according to whether there was a change in the participant's physical activity from 10-20 years prior to 1-10 years prior to breast cancer diagnosis. Compared to inactive patients, decreased or increased physical activity by >3 hours/week from 10-20 years prior to 1-10 years before diagnosis was not significantly associated with risk of all-cause mortality (data not shown). For breast cancer-specific mortality, there was a suggestion of an inverse association for lifetime physical activity; point estimates were similar to those for all-cause mortality. However, the sample size was smaller for breast cancer-specific deaths, and results were not statistically significant. Further, among postmenopausal women, lifetime physical activity was associated with decreased all-cause mortality (HR = 0.59, 95% CI, 0.35-0.99 and HR = 0.60, 95% CI, 0.36-0.99 for physical activity 3-6 and >6 hours/ week, respectively). The association of lifetime physical activity with all-cause mortality among premenopausal women was similar but the sample size was smaller and confidence intervals included the null (HR = 0.77, 95% CI, 0.33-1.79 and HR = 0.61, 95% CI, 0.24-1.54 for physical activity 3-6 and >6 hours/ week, respectively).

Among breast cancer patients with ER⁺ tumors, compared to inactive women, those women who engaged in average lifetime physical activity >6 hours/week had decreased risk of mortality from any cause (HR = 0.52, 95% CI 0.29-0.93; p trend = 0.03, Table 3). Adjusted HRs were similar for 1-10 and 10-20 years before diagnosis. Among women with ER⁻ tumors, associations were less strong and all confidence intervals included the null for both all-cause mortality and breast cancer-specific mortality. The interaction between lifetime physical activity and ER status in relation to all-cause mortality did not reach formal statistical significance (p interaction = 0.10). Lifetime average physical activity, physical activity 1-10 and 10-20 years before diagnosis were not significantly associated with risk of breast cancer-specific mortality for either ER⁺ or ER⁻ tumors.

Similarly, we observed slightly stronger inverse associations between lifetime physical activity and all-cause mortality among those with PR⁺ tumors as compared to those with PR⁻ tumors (HR = 0.45, 95% CI 0.24-0.87 for PR⁺ tumors and HR = 0.89, 95% CI 0.42-1.88 for PR⁻ tumors) (data not shown). We also examined associations stratified by HER2 status. Among women with HER2⁺ tumors, lifetime physical activity was inversely associated with risk of all-cause mortality (HR = 0.51, 95% CI 0.27-0.96 for physical activity >6 vs <3 hours/ week). Again, the inverse association of physical activity with all-cause mortality was observed among HER2⁻ breast cancer patients, but the results were not statistically significant and the sample size was small for all categories (data not shown).

Results for analyses of all-cause mortality stratified by tumor *TP53* mutation status are shown in Table 4. Among women with tumors with a *TP53* mutation, lifetime physical activity was strongly inversely associated with risk of all-cause mortality (HR = 0.22, 95% CI, 0.06-0.72, for >6 hours/ week vs <3 hours/ week, p trend = 0.01). Among women with tumors without *TP53* mutations, no association between lifetime physical activity and death was observed. However, the difference in the associations in the groups defined by *TP53* status did not reach formal statistical significance; there was no statistical interaction between lifetime physical activity and *TP53* mutation status in relation to all-cause mortality (p interaction = 0.11). In analyses of breast cancer-specific mortality, there were no clear associations of any of these measurements of physical activity across *TP53* mutation status (data not shown).

Discussion

We examined the association of physical activity prior to diagnosis with breast cancer survival, including an examination of differences in associations across tumor characteristics. A number of previous studies have investigated the association between physical activity prior to breast cancer diagnosis and the risk of overall or breast cancer-specific mortality [2, 9-11, 32-36]. Although there are differences in physical activity measurement, sample size, follow-up periods or other differences in the population under study, these studies have shown a relatively consistent association between pre-diagnostic physical activity and improved breast cancer prognosis.

In our study, lifetime physical activity, physical activity 1-10 years and 10-20 years prior to diagnosis was associated with decreased risk of all-cause mortality. Our results are consistent with those previous studies [2, 9, 10, 33-35], but they are not consistent with the findings of some other studies [11, 36]. In addition, results from two studies suggested that recent prediagnostic physical activity may have stronger protective effect on mortality risk than activity undertaken in the distant past [2, 33]. However, we did not see stronger associations for recent activity; our results showed similar risk reductions in mortality associated with lifetime and more recent (1-10 and 10-20 years prior to the breast cancer diagnosis) physical activity. We further found a similar inverse association among women who were consistently active both 10-20 years before and 1-10 years before breast cancer diagnosis, while there was no significant association with decreased or increased physical activity from 10-20 years prior to 1-10 years before diagnosis. Reports of physical activity very close to the time of diagnosis may reflect changes related to the disease, and disease status could affect reports of lifetime physical activity, especially among those with later stage disease. Some studies have suggested that recent prediagnostic physical activity may correlate with postdiagnostic activity levels [2]. It is unclear whether breast cancer survival would be affected by change in physical activity at particular time periods, and whether pre- and post-diagnostic physical activity impacts breast cancer survival separately or continuously. Further studies are needed to elucidate if there is an time period which is most critical for physical activity with respect to breast cancer survival.

Physical activity might influence breast cancer survival distinctly for different subtypes. A few studies have examined the relationship of prediagnostic physical activity with breast cancer survival stratified by ER status [9-11]. Keegan et al [11] reported a strong inverse association between recreational activity during the three years prior to breast cancer diagnosis and risk of all-cause mortality limited to those with ER⁺ tumors. In other studies, there have not been differences by ER status [9, 10]. In our study, we found somewhat stronger inverse associations of lifetime physical activity and overall mortality among ER⁺ tumors than ER⁻ tumors. The inverse relationship between prediagnostic physical activity and survival may involve reduced endogenous estrogen among those who are more physically active because of either the impacts of physical activity on adipose tissue accumulation or its impact on premenopausal menstrual function, especially among women with hormone-responsive tumors [37].

To our knowledge, only one epidemiological study has examined the interaction between p53 protein histochemical detection and physical activity with regard to cancer survival. Meyerhardt et al. [38] assessed protein expression of p53 among 484 early stage colon cancer patients. Although no significant interaction was detected between p53 status and physical activity, a stronger association between post diagnosis physical activity and decreased risk of overall mortality was found among colon cancer patients with higher p53 expression (HR = 0.44, 95% CI, 0.24-0.80 for exercise metabolic equivalent (MET)-hours/week vs <18 MET-hours/week) than those with low p53 expression (HR = 0.73, 95% CI,

0.45-1.19). Our study examined prediagnostic physical activity and focused on *TP53* mutation status, and our results appeared consistent with those of the study of colon cancer patients. We observed a stronger inverse association between high physical activity and overall mortality among breast cancer cases with tumors carrying a *TP53* mutation, although the difference in the association of physical activity and survival by *TP53* mutation status did not reach statistical significance for interaction. These results suggested a potential combined effect between physical activity and altered p53 status. More studies with larger sample size are needed to replicate our findings and to further assess the influences of other molecular features of tumor on the association of physical activity and breast cancer survival.

In addition to effects on the hormonal milieu, physical activity may also contribute to improved survival through influencing insulin resistance and inflammation pathways [39-41]. *In vivo* studies have shown that moderate intensity aerobic physical activity lowers serum insulin and insulin-like growth factor (IGF) and increases IGF binding protein (IGFBP-1), which are associated with reduction in cancer cell growth and survival [42]. A recent study investigated the effects of aerobic exercise on tumor physiology in an animal model of human breast cancer, and found that intratumoral perfusion/vascularization and hypoxia were significantly higher in the exercise group than the sedentary control group [43], leading to “normalization” of the tumor tissue microenvironment and inhibition of metastasis.

There is evidence of *TP53* mutations in breast ductal carcinoma *in situ* and in breast atypical hyperplasia [44, 45], suggesting the mutation may occur as an early event in breast carcinogenesis. Mutation of *TP53* and the resulting p53 inactivation may modify the bioenergetics profile of cancer cells, making them more sensitive to the effects of physical activity and thus contributing to reduce the risk of recurrence and dissemination. The inactivation of p53 by mutation contributes to dependence of cells upon glycolysis as main source of energy, decreases mitochondrial biogenesis and impairs respiratory capacity [16]. Furthermore, loss of p53 function may remove a critical component of cell response to excess production of damaging and reactive oxygen species [16]. Thus, when facing exposure to high oxygen through aerobic exercise, cells with mutant *TP53* may be sensitive to metabolic stress, leading to increased mitochondrial leakage and high levels of oxyradical and mitochondrial components such as cytochrome c. Physical activity may operate as a possible biological mechanism that stimulates an endogenous pathway for cell death, and then protect against breast cancer mortality. However, the role of p53 in the relationships between exercise, mitochondrial respiration, cancer cell growth and tumor prognosis remains to be elucidated. Nonetheless, the possibility remains that the stronger associations among women with *TP53* mutation are by chance alone. Further studies in other populations are needed to confirm our findings.

In assessing these findings, it is important to consider strengths and weakness of the study. Strengths include the population-based patient cohort, the prospectively collected detailed information on lifetime physical activity, cancer characteristics and treatments, and relatively long follow-up period. However, our study has several limitations. Although cases were interviewed shortly after their diagnosis in our study, some eligible cases might have died before they could be enrolled in the study. However, for breast cancer, this is likely a small percent of the total. In our study, the 1- and 5-year overall breast cancer survival is 99.6% and 90.9%. Using this method, we limit survival bias with regard to that cohort, although generalization of our results to all breast cancer cases diagnosed in the study region, particularly those with later stage tumors may be somewhat limited. There may have been a bias in the recall of lifetime self-reported physical activity. Further, those with later stage disease may have reduced their physical activity even before the diagnosis in response

to the disease and that change might impact their report of lifetime activity. However, the inverse association with prediagnostic physical activity was similar across different strata of the stage. In addition, missing data on ER or *TP53* mutation status in a sizable fraction of study subjects may also be a concern. In comparisons of cases with the information on these tumor characteristics, cases without the information were more likely to have tumors of earlier stage; but the two groups were similar in terms of age at diagnosis, BMI, average lifetime physical activity, and tumor size. Lastly, information on physical activity changes after cancer treatment was not available for our study subjects; we were unable to evaluate the relationship between physical activity changes after diagnosis and breast cancer mortality by *TP53* mutation status.

In summary, our results contribute to the growing body of evidence that prediagnostic physical activity is associated with decreased risk of all-cause mortality in breast cancer patients. Our findings indicate that risk reduction in mortality may be stronger among tumors that are ER⁺ or have a *TP53* mutation. Although the biologic mechanisms underlying the associations require to be further investigated, these findings support the idea that cancer cells develop specific metabolic adaptations, which may be adequately targeted by physical activity. Further studies are needed to better understand the different effects of physical activity on patient survival by molecular features of the tumor; however, physical activity prior to diagnosis appears to be a beneficial strategy to reduce risk of mortality among breast cancer survivors.

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Table 1Descriptive characteristics of breast cancer cases by vital status, WEB Study¹

	Alive as of December 2006 (n= 1000)	Died as of December 2006 (n = 170)
Age at diagnosis (yrs)	57.0 (10.9)	59.7 (12.3) **
Body mass index (kg/m ²)	28.3 (6.2)	29.5 (6.7) *
Age at menarche (yrs)	12.6 (1.6)	12.6 (1.6)
Age at first birth (yrs)	20.0 (10.3)	19.6 (9.3)
Age at menopause (yrs) ¹	48.4 (5.3)	47.6 (6.0)
Race		
Caucasian	928 (92.8)	149 (87.6) *
Other	72 (7.2)	21 (12.4)
Education (yr)		
<12	76 (7.6)	21 (12.4) **
12	369 (36.9)	76 (44.7)
>12	555 (55.5)	73 (42.9)
Menopausal status		
Premenopausal	281 (28.1)	45 (26.5)
Postmenopausal	719 (71.9)	125 (73.5)
TNM		
0	136 (13.6)	10 (5.9) **
I	440 (44.0)	46 (27.1)
II _a	187 (18.7)	25 (14.7)
II _b	74 (7.4)	30 (17.6)
III-IV	31 (3.1)	30 (17.6)
Unknown	132 (13.2)	29 (17.1)
Radiotherapy ²		
Received or planned	656 (67.8)	93 (59.2) *
No	312 (32.2)	64 (40.8)
Chemotherapy ²		
Yes	348 (35.7)	92 (55.4) **
No	627 (64.3)	74 (44.6)
Hormonal therapy ²		
Ever	565 (59.2)	74 (48.1) **
No	390 (40.8)	80 (51.9)
ER status		
Positive	664 (66.4)	88 (51.8) **
Negative	245 (24.5)	70 (41.2)
Unknown	91 (9.1)	12 (7.0)

	Alive as of December 2006 (n= 1000)	Died as of December 2006 (n = 170)
PR status		
Positive	580 (58.0)	76 (44.7) **
Negative	311 (31.1)	81 (47.7)
Unknown	109 (10.9)	13 (7.6)
TP53 mutation status		
Wild type	456 (45.6)	72 (42.3) **
Mutant	160 (16.0)	45 (26.5)
Unknown	384 (38.4)	53 (31.2)
HER2		
Negative	584 (58.4)	93 (54.7)
Positive	59 (5.9)	15 (8.8)
Unknown	357 (35.7)	62 (36.5)

Data are shown as Means $\pm$ S.D. (continuous variables) or n (%) (categorical variables).

¹ Among postmenopausal women

² Subjects with missing values were excluded from the analysis.

* $P < 0.05$

** $P < 0.01$.

Table 2

All-cause mortality and breast cancer specific mortality after diagnosis of breast cancer, by physical activity
WEB study

	All-cause mortality		Breast cancer-specific mortality	
	Deaths/cohort	HR (95%CI) ^a	Deaths/cohort	HR (95%CI) ^a
Average lifetime physical activity (hours/week)				
<3	45/254	1.0	27/254	1.0
3-6	61/469	0.64 (0.42-1.00)	38/469	0.64 (0.36-1.13)
>6	64/447	0.61 (0.40-0.95)	35/447	0.62 (0.34-1.11)
<i>P</i> trend		0.04		0.14
Percentage of time being more active (>3 hours/week) in lifetime (%)				
<25	34/193	1.0	19/193	1.0
25-49	17/126	0.89 (0.47-1.72)	12/126	1.06 (0.47-2.35)
50-74	31/173	1.07 (0.62-1.84)	18/173	0.83 (0.40-1.75)
75	88/678	0.60 (0.39-0.95)	51/678	0.67 (0.37-1.22)
<i>P</i> trend		0.02		0.13
Average physical activity 1-10 yrs prior (hours/week)				
<3	70/404	1.0	37/404	1.0
3-6	41/352	0.56 (0.37-0.86)	29/352	0.71 (0.41-1.23)
>6	59/414	0.73 (0.50-1.08)	34/414	0.86 (0.50-1.48)
<i>P</i> trend		0.11		0.60
Average physical activity 10-20 yrs prior (hours/week)				
<3	63/351	1.0	34/351	1.0
3-6	36/348	0.52 (0.33-0.81)	25/348	0.71 (0.39-1.28)
>6	71/471	0.70 (0.47-1.03)	41/471	0.83 (0.49-1.42)
<i>P</i> trend		0.11		0.59

^a Adjusted for age at diagnosis (continuous variable), race (white, others), education (< 12 yrs, 12 yrs, > 12 yrs), BMI (<25, 25-29, ≥30), menopausal status, TNM (0, I, IIa, IIb, III-IV, unknown), radiotherapy (yes, no), chemotherapy (yes, no), hormonal therapy (yes, no), *p53* mutation (yes, no, unknown), Her2 status (negative, positive, unknown), ER status (positive, negative, unknown), and PR status (positive, negative, unknown).

Table 3
All-cause mortality and breast cancer specific mortality after diagnosis of breast cancer across ER status, by physical activity WEB study

	All-cause mortality				Breast cancer-specific mortality			
	ER ⁺		ER ⁻		ER ⁺		ER ⁻	
	Deaths/cohort	HR (95%CI) ^a	Deaths/cohort	HR (95%CI) ^a	Deaths/cohort	HR (95%CI) ^a	Deaths/cohort	HR (95%CI) ^a
Average lifetime physical activity (hours/week)								
<3	26/169	1.0	15/53	1.0	13/169	1.0	10/53	1.0
3-6	27/298	0.55 (0.30-0.99)	32/141	0.98 (0.44-2.19)	12/298	0.41 (0.17-1.00)	25/141	1.18 (0.47-2.98)
>6	35/285	0.52 (0.29-0.93)	23/121	0.69 (0.28-1.70)	19/285	0.58 (0.25-1.34)	15/121	0.70 (0.24-2.02)
P trend		0.03		0.32		0.25		0.37
Percentage of time being more active (>3 hours/week) in lifetime (%)								
<25	19/128	1.0	12/43	1.0	8/128	1.0	8/43	1.0
25-49	9/87	0.87 (0.36-2.05)	7/26	1.38 (0.39-4.81)	5/87	1.05 (0.33-3.32)	6/26	1.39 (0.34-5.72)
50-74	14/114	0.78 (0.36-1.67)	15/47	1.66 (0.65-4.23)	7/114	0.58 (0.17-1.95)	10/47	1.26 (0.42-3.74)
75	46/423	0.52 (0.29-0.94)	36/199	0.72 (0.30-1.76)	24/423	0.67 (0.28-1.64)	26/199	0.76 (0.26-2.18)
P trend		0.02		0.25		0.34		0.43
Average physical activity 1-10 yrs prior (hours/week)								
<3	39/270	1.0	24/87	1.0	16/270	1.0	16/87	1.0
3-6	20/216	0.50 (0.27-0.91)	21/112	0.83 (0.41-1.67)	11/216	0.52 (0.21-1.27)	18/112	0.95 (0.42-2.16)
>6	29/266	0.57 (0.33-0.99)	25/116	0.88 (0.44-1.79)	17/266	0.87 (0.39-1.96)	16/116	0.76 (0.31-1.83)
P trend		0.04		0.76		0.82		0.53
Average physical activity 10-20 yrs prior (hours/week)								
<3	34/239	1.0	23/75	1.0	14/239	1.0	15/75	1.0
3-6	19/215	0.51 (0.28-0.94)	17/104	0.74 (0.34-1.60)	11/215	0.68 (0.27-1.70)	14/104	1.10 (0.44-2.79)
>6	35/298	0.55 (0.32-0.95)	30/136	0.82 (0.41-1.66)	19/298	0.80 (0.35-1.83)	21/136	0.98 (0.41-2.33)
P trend		0.04		0.68		0.66		0.92

^a Adjusted for age at diagnosis (continuous variable), race (white, others), education (< 12 yrs, 12 yrs, > 12 yrs), BMI (<25, 25-29, 30), menopausal status, TNM (0, I, IIa, IIb, III-IV, unknown), radiotherapy (yes, no), chemotherapy (yes, no), hormonal therapy (yes, no, unknown), Her2 status (negative, positive, unknown), and PR status (positive, negative, unknown).

Table 4

Hazards ratios of all-cause mortality after diagnosis of breast cancer across *TP53* mutation status, by physical activity WEB study

	<i>TP53</i> mutation status			
	Wild type		Mutant	
	Deaths/cohort	HR (95%CI) ^a	Deaths/cohort	HR (95%CI) ^a
Average lifetime physical activity (hours/week)				
<3	18/106	1.0	11/44	1.0
3-6	23/215	0.51 (0.24-1.09)	21/93	0.52 (0.19-1.42)
>6	31/207	0.74 (0.37-1.48)	13/68	0.22 (0.06-0.72)
<i>P</i> trend		0.64		0.01
Percentage of time being more active (>3 hours/week) in lifetime (%)				
<25	13/85	1.0	9/31	1.0
25-49	8/56	1.00 (0.34-2.91)	4/21	0.52 (0.11-2.38)
50-74	11/79	0.79 (0.29-2.15)	9/35	0.49 (0.14-1.69)
75	40/308	0.79 (0.38-1.64)	23/118	0.32 (0.10-0.98)
<i>P</i> trend		0.47		0.05
Average physical activity 1-10 yrs prior (hours/week)				
<3	27/175	1.0	17/64	1.0
3-6	19/170	0.78 (0.39-1.56)	14/71	0.71 (0.28-1.79)
>6	26/183	1.00 (0.54-1.85)	14/70	0.56 (0.21-1.49)
<i>P</i> trend		0.98		0.25
Average physical activity 10-20 yrs prior (hours/week)				
<3	26/155	1.0	16/57	1.0
3-6	13/163	0.48 (0.22-1.02)	13/69	0.39 (0.14-1.11)
>6	33/210	0.96 (0.53-1.76)	16/79	0.37 (0.13-0.99)
<i>P</i> trend		0.97		0.07

^a Adjusted for age at diagnosis (continuous variable), race (white, others), education (< 12 yrs, 12 yrs, > 12 yrs), BMI (<25, 25-29, ≥30), menopausal status, TNM (0, I, IIa, IIb, III-IV, unknown), radiotherapy (yes, no), chemotherapy (yes, no), hormonal therapy (yes, no), Her2 status (negative, positive, unknown), ER status (positive, negative, unknown), and PR status (positive, negative, unknown).