Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

Research Article

Cancer Prevention Research

Uninterrupted Sedentary Behavior Downregulates *BRCA1* Gene Expression

Rachael Pettapiece-Phillips[1,2], Max Kotlyar[3], Rania Chehade[1], Leonardo Salmena[3,4], Steven A. Narod[1,2,5], Mohammad Akbari[1,5], Igor Jurisica[3,6], and Joanne Kotsopoulos[1,2,5]

Abstract

BRCA1 mutation carriers face a high lifetime risk of developing breast cancer. Physical activity induces broad transcriptional changes, and multiple studies have documented its beneficial effects across cancers. Because haploinsufficiency predisposes to breast cancer in these women, factors that increase *BRCA1* levels may mitigate the effect of the mutation. Whether physical activity modulates *BRCA1* expression and whether lifestyle factors could benefit women with a mutation remain unclear. The objective of this study was to systematically evaluate whether physical activity or sedentary behavior affects *BRCA1* mRNA expression. Activity levels were assessed in 50 female participants (14 *BRCA1* mutation carriers and 36 noncarriers) using the GT3X Actigraph accelerometer, and *BRCA1* mRNA expression was quantified from peripheral blood lymphocytes using the Nanostring nCounter Analysis System. There was a significant negative correlation between the longest sedentary bout and *BRCA1* mRNA expression ($\rho = -0.32$; $P = 0.02$). Women below the median for the longest sedentary bout had significantly higher *BRCA1* mRNA levels compared with women above the median (161 vs. 132 counts; $P = 0.04$; one-sided Mann–Whitney *U* test). There was no significant relationship between mean metabolic equivalents of task rate or mean sedentary time and *BRCA1* mRNA expression (Spearman correlation $P \geq 0.75$; $P \geq 0.14$; Mann–Whitney *U* test). These findings suggest that prolonged periods of sedentary behavior are associated with significantly lower *BRCA1* mRNA expression. Whether this translates into a potentially more harmful effect in *BRCA1* mutation carriers warrants further investigation. *Cancer Prev Res; 9(1); 83–88. ©2015 AACR.*

Introduction

Women with a *BRCA1* mutation face an elevated risk of developing breast cancer, estimated at 60% by age 70 (1–3). Despite the well-established protective effects of preventive surgery, whether or not lifestyle factors influence the development and/or progression of *BRCA*-associated cancer remains unclear (4). Epidemiologic evidence supports a protective role of physical activity in the development of breast cancer among women at average risk (5–7). Of increasing importance is the independent adverse role of sedentary behavior (defined as sitting time) on chronic disease (8, 9). Only a few studies have evaluated whether such an association exists among women with a *BRCA1* mutation (10–13). Cancer-protective mechanisms of physical activity have mainly focused on alterations in levels of sex hormones, inflammatory markers, and growth factors (14–16). It is plausible that such changes are due to physical activity–induced transcriptional changes, as physical activity has been shown to induce widespread transcriptional effects (17–20). This may be of particular relevance for *BRCA1* mutation carriers given that their cancer predisposition is likely the result of haploinsufficiency associated with heterozygosity, thereby increasing genomic instability and accelerating the mutation rate of other critical genes (including the second copy of *BRCA1*; ref. 21). Of particular interest is an *in vivo* study showing that physical activity resulted in a significant increase in *BRCA1* mRNA expression in the mammary glands of exercised compared with unexercised rats (22).

Thus, the overall goal of the current study was to systematically evaluate the relationship between measures of physical activity and sedentary behavior with *BRCA1* mRNA expression. Factors that might increase the physiologic expression of the normal copy of *BRCA1* and normalize protein levels may mitigate the effect of an inherited *BRCA1* mutation, representing a plausible mechanism whereby these exposures may modify cancer risk in *BRCA1* mutation carriers. To the best of our knowledge, ours is the first study evaluating such an association in *BRCA1* mutation carriers.

Materials and Methods

Study design and population

Subjects included women who were at least 18 years old, had no personal history of cancer, and who were not pregnant or breastfeeding. The first group included women from the general population, recruited via posters, newsletters, or social media. The second group included women with a confirmed *BRCA1* mutation, identified from the Women's College Research Institute

[1]Women's College Research Institute, Women's College Hospital, Toronto, Ontario, Canada. [2]Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada. [3]Princess Margaret Cancer Centre, University Health Network, IBM Life Sciences Discovery Centre, Toronto, Ontario, Canada. [4]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada. [5]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada. [6]Department of Medical Biophysics and Department of Computer Science, University of Toronto, Toronto, Ontario, Canada.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

R. Pettapiece-Phillips and M. Kotlyar share first authorship.

Corresponding Author: Joanne Kotsopoulos, University of Toronto, 76 Grenville Street, 6th Floor, Toronto, ON M5S 1B2, Canada. Phone: 416-351-3800; Fax: 416-351-3767; E-mail: joanne.kotsopoulos@wchospital.ca

doi: 10.1158/1940-6207.CAPR-15-0291

Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

(WCRI, Toronto, Canada). Two 30-minute appointments were scheduled for participants to provide study details. The study protocol was approved by the Research Ethics Board at the Women's College Hospital (# 2012-0055-B). All patients provided written-informed consent, and a total of 50 women enrolled in the study: 14 *BRCA1* mutation carriers and 36 noncarriers.

Data and biologic sample collection

At the first visit, participants completed a research questionnaire to collect information on reproductive and medical history, lifestyle factors, and family history of cancer. Standardized procedures were used to collect anthropometric measurements [i.e., weight (kg), height (m), waist (inches), and hip circumference (inches)]. Measurements were taken in duplicate, and an average of the two measures was calculated. The four measurements were used to calculate body mass index (BMI; kg/m^2), waist-to-hip-ratio (WHR), and the index of central obesity [waist circumference (inches)/height (inches)]. At the second study visit, a phlebotomist drew blood into two labeled EDTA containing tubes (~8 mL) by venipuncture. The samples were placed on ice and delivered within 35 minutes to the Narod laboratory (Women's College Hospital) for RNA extraction.

Physical activity assessment

All participants were provided with a detailed explanation on how to use the ActiGraph GT3X accelerometer (ActiGraph). Participants were asked to wear the accelerometer on an elasticized belt for 7 consecutive days during waking hours except during bathing and water activities. The GT3X is a triaxial monitor that detects movement in vertical, horizontal, and anterior–posterior planes and converts these data into counts, which are used to calculate energy expenditure (23). Each monitor was programmed to record activity information for 1-second epochs. The GT3X monitor has been validated for use in an adult population (24). At the second study visit, participants returned the accelerometer and data from the GT3X were downloaded onto a secure server.

For the current analysis, a valid day was defined as at least 600 minutes of accelerometer wear. "Nonwear" time was defined as an interval of at least 10 consecutive minutes of zero activity counts. This resulted in all 50 participants having 6 or more valid days of monitor wear. "Wear-time" was validated using cut points from the Troiano and colleagues study (25). Raw accelerometer counts were used to determine the percentage of time each participant spent in various activity levels (i.e., sedentary, light, and moderate to vigorous) using the manufacturer's software (ActiLife ver. 6.8.2) in conjunction with previously described cut points (hours/day; ref. 25). The percentage time was calculated by adding the minutes within each activity level and then dividing by the total minutes of wear per day. The metabolic equivalents of task (MET) rate were calculated using the Swartz Adult Overground and Lifestyle cut points (26). METs are an estimation of the energy cost of physical activity defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1.0 ($4.184\ kJ \cdot kg^{-1} \cdot h^{-1}$). MET values range between 0 and 23, while a value of 1 MET represents resting metabolic rate during quiet sitting and 18 represents running at 16.1 kmph (27). The longest sedentary bout was defined as the length of longest inactivity time (seconds) within the 7 consecutive days of accelerometer wear. Mean sedentary time (hours/day) was defined as the average amount of time per day spent being sedentary.

RNA quantification

RNA isolation. RNA was isolated from one of the two EDTA tubes using the LeukoLOCK Total RNA Isolation System (Ambion; ref. 28). In order to maximize RNA isolation yield, all samples were stabilized with RNA within 35 minutes of blood draw. Resulting extracted RNA was stored in several aliquots at −80°C.

RNA yield and quality. Two aliquots per participant were used to assess the quality and quantity of RNA. The nucleic acid content was quantified using the Nanodrop spectrophotometer (ThermoScientific). Total RNA quality and quantity were then determined using the Agilent 2100 Bioanalyzer (The Centre for Applied Genomics, Toronto, Canada). The mean sample RNA concentration was 173.7 ng/μL (range, 85–368 ng/μL), which is sufficient for downstream analyses (29). The mean RNA integrity number (RIN) for the samples of interest was 8.7 (range, 7.9–9.3; refs. 30, 31).

Quantification of BRCA1 mRNA

The nCounter Human Cancer Reference Kit consisting of 230 cancer-related genes (Nanostring Technologies) was used to measure *BRCA1* mRNA expression (expressed as counts) at the University Health Network Genomics Centre (Toronto, Canada; ref. 29). Briefly, the nCounter Analysis System probe library contains two sequence-specific probes, the capture probe and the reporter probe, for each gene of interest. Probe pairs are mixed with total RNA in one hybridization reaction, and then the structures are imaged with the use of fluorescent microscopy. Expression is measured by counting the number of unique colour tags within the gene-probe tripartite structures and is reported as "counts."

Data analysis was performed using the nSolver software (Nanostring Technologies) and custom scripts in R (ver. 3.1.2). Positive and negative controls were used to check for background expression. Reference housekeeping gene normalization was then performed. As the aim of our study was to investigate the effects of physical and sedentary activity on *BRCA1* expression, we strove to ensure that our set of reference genes was minimally affected by physical and sedentary activities. This was especially important for investigating the longest sedentary bouts, which were negatively correlated with the expression of 89% (211 of 236) of measured genes. To identify a reference gene set unaffected by longest sedentary bouts, we randomly sampled 5,000 sets of five genes; for each set, we calculated its geometric mean in each sample and then calculated the Spearman correlation between the means and longest sedentary bouts. We chose the set with the lowest correlation magnitude (closest to 0) as our reference set. This set comprised the following genes: *CCND3* (cyclin D3), *CDKN2B* (cyclin-dependent kinase inhibitor 2B), *COL1A1* (collagen, type I, alpha 1), *MMP1* (matrix metallopeptidase 1), *TNFSF10* [tumor necrosis factor (ligand) superfamily, member 10]. The geometric mean of these five genes was calculated for each lane, and the average of the geometric means was calculated across all lanes. A lane-specific normalization factor was calculated for each lane by dividing the average by the geometric mean of the lane. The endogenous counts in each lane were then multiplied by the lane's normalization factor (32).

Statistical analysis

The effects of physical activity and sedentary time on *BRCA1* mRNA expression were assessed by Mann–Whitney *U* test,

Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

correlation, and linear regression. Dichotomous categories (high/low) of the longest sedentary bout (seconds), mean sedentary time (hours), and mean MET rate (kcal/kg/hour) were created, using the median to define high (above the median) versus low (at or below the median) levels for each of the exposures. The Mann–Whitney *U* test was used to evaluate differences in median *BRCA1* mRNA levels among women in the high versus low exposure categories. Spearman correlation was calculated between each exposure and *BRCA1* mRNA expression. Linear regression was used to examine the influence of physical activity and sedentary behavior on *BRCA1* mRNA expression, adjusting for age (years), *BRCA1* mutation status (carrier/noncarrier), and BMI (continuous).

Statistical significance was defined at the level of $P \leq 0.05$, and all analyses were carried out using R Version 3.1.2.

Results

Table 1 summarizes selected participant characteristics. The average age of the participants was 37.2 years, and thus, the majority of the women were premenopausal (74%) and nulliparous (60%). The mean BMI of the participants was 24.6, which is at the high end of the normal weight range defined as a BMI of 18.50 to 24.99.

Table 1. Selected characteristics of study participants

Characteristic	All ($n = 50$)
Age (years), mean, range	37.2 (18–62)
Ethnicity, *n* (%)	
Other white	25 (50%)
Ashkenazi Jewish	12 (24%)
Hispanic	3 (6%)
East Asian	6 (12%)
South Asian	4 (8%)
Parous, *n* (%)	20 (40%)
Age at menarche (years), mean (SD)	12.4 (1.3)
Postmenopausal, *n* (%)	13 (26%)
Current oral contraceptive use, yes, *n* (%)	6 (12%)
Current smoking status, yes, *n* (%)	1 (2%)
Current alcohol consumption, yes, *n* (%)	43 (86%)
Mastectomy, yes, *n* (%)	3 (6%)
Oophorectomy, yes, *n* (%)	9 (18%)
Current hormone replacement therapy use, *n* (%)	8 (16%)
Physical activity/inactivity variables (per day)	
Sedentary time (hours), mean (SD)	8.60 (1.5)
Percentage of time in sedentary mode, *n* (%)	
<60%	13 (26)
60%–70%	23 (46)
>70%	14 (28)
Longest sedentary bout (seconds), mean (SD)	7,158 (3,854)
MET rate, mean (SD)	1.83 (0.2)
MVPA (min), mean (SD)	44.9 (21.7)
Percentage of time in MVPA, *n* (%)	
<5%	22 (44)
5%–8%	19 (38)
>8%	9 (18)
Body size variables	
BMI, kg/m^2 (SD)	24.6 (5)
Normal (19.0–24.99; %)	29 (58)
Overweight (25.0–29.99; %)	14 (28)
Obese (>30; %)	7 (14)
Waist circumference, inches (SD)	33.2 (4.8)
Hip circumference, inches (SD)	39.1 (4.1)
WHR, (SD)	0.85 (0.06)
Index of central obesity, (SD)[a]	0.52 (0.07)

Abbreviation: MVPA, Moderate to vigorous physical activity.
[a]Index of central obesity = wait circumference (inches)/height (inches).

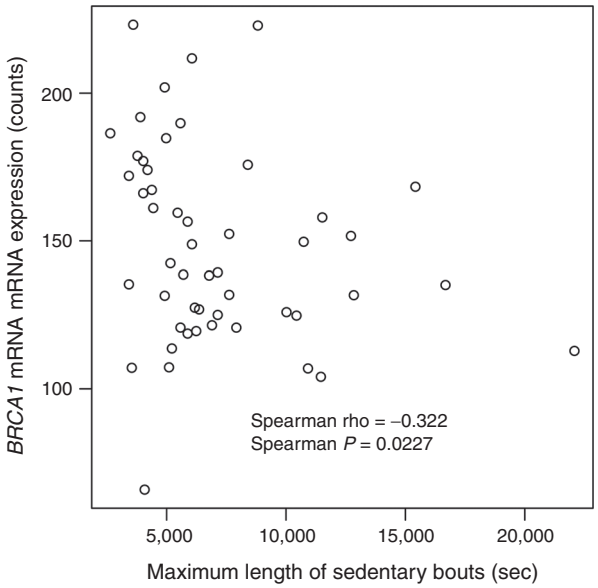

Figure 1.
Correlation between the longest sedentary bout (seconds) and *BRCA1* mRNA expression (counts).

The mean daily sedentary time was 8.60 hours per day, and 28% of the women spent more than 70% of their time in sedentary mode. The mean maximum length of a sedentary bout was 7,158 seconds (SD = 3854). There was a significant negative correlation between the longest sedentary bout (seconds) and *BRCA1* mRNA expression (counts; $\rho = -0.32$; $P = 0.02$; Fig. 1). The relationship remained statistically significant ($\rho = -0.28$; $P = 0.049$) when adjusted for age, *BRCA1* mutation status, and BMI (data not shown). Figure 2 illustrates that women below the median of the longest sedentary bout had significantly higher *BRCA1* mRNA levels compared with women above the median (161 vs. 132 counts; $P = 0.037$; one-sided Mann–Whitney *U* test). There was no significant relationship between the mean MET rate (kcal/kg/hour) and *BRCA1* mRNA expression (Supplementary Figs. S1 and S2). In an analysis stratified by *BRCA1* mutation status, the negative correlation between the longest sedentary bout and *BRCA1* mRNA expression was stronger for *BRCA1* mutation carriers; however, this association did not achieve statistical significance likely due to the small sample size ($\rho = -0.45$; $P = 0.11$ vs. $\rho = -0.21$; $P = 0.22$ in noncarriers).

We also observed no significant correlation between mean sedentary time (hours/day) and *BRCA1* mRNA expression ($\rho = -0.045$; $P = 0.75$; Supplementary Fig. S3). There was no significant difference in *BRCA1* mRNA levels between women with mean sedentary time above compared with below the median (142 vs. 139 counts; $P = 0.73$; one-sided Mann–Whitney *U* test; Supplementary Fig. S4).

To systematically analyze what processes and signaling cascades involve genes significantly deregulated by sedentary activity ($n = 24$; Supplementary Table S1), we used pathDIP ver. 1 (33). pathDIP provides literature-driven and computationally predicted annotations for known membership and significant association of genes to molecular pathways. It

Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

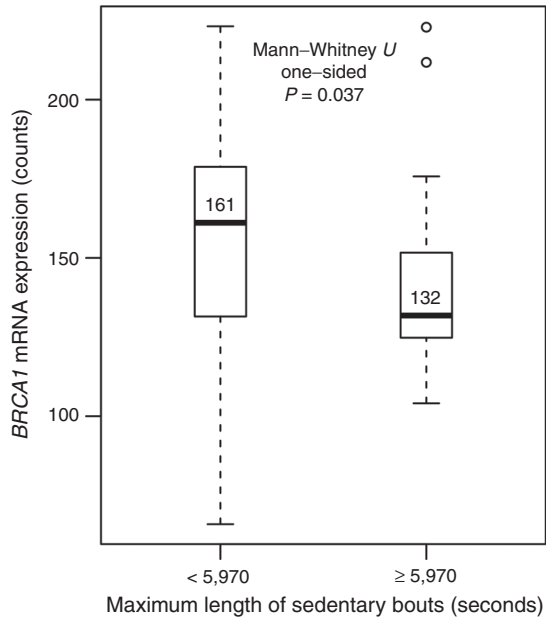

Figure 2.
Median *BRCA1* mRNA expression (counts) among women above versus below the median for the longest sedentary bout (seconds).

integrates data that originate from multiple resources for molecular pathway annotations (34) and protein–protein interactions (35, 36). This analysis identified 968 significantly enriched pathways ($P < 0.01$; list in Supplementary Table S2). Significantly enriched pathways included diseases: cancer; processes: apoptosis, cell-cycle, and DNA damage response; and proteins and signaling cascades: MAPK, NF-κB, WNT, and PI3K.

Discussion

The overall goal of this study was to evaluate whether accelerometer-derived measures of physical activity and sedentary behavior are associated with *BRCA1* expression. We observed a significant negative correlation between the longest sedentary bout and *BRCA1* mRNA levels but no relationship with MET rate. These findings point toward perhaps an even more important role of sedentary behavior *per se* rather than physical activity influencing gene expression. Importantly, because no significant relationship with mean sedentary time was observed, prolonged periods of inactivity may have a larger impact on *BRCA1* rather than shorter periods of sedentary time with more frequent interruptions. These findings are in line with current literature outlining the detrimental and possibly additive effects of sedentary time, physical inactivity, and television watching on chronic disease (8, 37–39). Nonetheless, given the multiple comparisons and relatively small sample size, these findings should be interpreted with caution and require replication in a larger study population. However, considering current World Health Organization recommendations of 150 minutes of physical activity per week for adults, our study strongly suggests that being active may not be sufficient in the presence of prolonged sedentary bouts (40).

When identifying lifestyle interventions that may prevent breast cancer, it is important to consider that the mechanism underlying the predisposition is likely to be different in *BRCA1* mutation carriers versus the general population (21). *BRCA1* is an established mediator of genomic stability and regulator of the cellular response to DNA damage (41). In these roles, *BRCA1* regulates processes pertinent to cell survival, proliferation, and differentiation (42). The consequences associated with inheritance of a single functional copy of the gene suggest a dosage effect of *BRCA1*. Therefore, titrating of *BRCA1* mRNA and protein levels through nongenetic mechanisms may also affect the normal functions of the gene. More recently, our group demonstrated that oral supplementation with the phytochemical 3,3′-diindolylmethane was associated with an increase in *BRCA1* mRNA expression in women with a *BRCA1* mutation, providing important preliminary evidence that nongenetic exposures may affect gene expression (43). Whether this translates to a change in protein levels and subsequent cancer risk is not yet known.

The current study was prompted by two earlier publications reporting a relationship between physical activity and *BRCA1* gene expression (22, 44). Using an *in vivo* model, Wang and colleagues found that prepubertal physical activity was associated with significantly higher mammary gland expression of *BRCA1* mRNA, among other genes, compared with unexercised rats (22). In the second study, Magbanua and colleagues reported that vigorous activity significantly upregulated several gene sets, including homologous recombination repair and double-strand break repair in prostate tissue of men undergoing active surveillance (44). Although *BRCA1* expression was not independently upregulated, both DNA repair pathways included *BRCA1* (among other genes), suggesting that vigorous physical activity may modulate genomic stability by affecting the response of the cell to DNA damage in the prostate.

Three epidemiologic studies have evaluated the effect of physical activity on *BRCA*-associated cancer development (10). King and colleagues reported a significant delay in breast cancer onset among *BRCA1/2* mutation carriers who were physically active as teenagers compared with those who were not (11), and Pijpe and colleagues reported a 42% reduction in risk with increasing levels of physical activity prior to, but not after, age 30 (HR, 0.58; 95% confidence interval, 0.35–0.94; highest vs. lowest tertile of mean MET hours/week before age 30; ref. 13). In contrast, a third study found no association between levels of physical activity (45). Although retrospective, these studies provide some evidence for a protective role of physical activity during adolescence or early adulthood among *BRCA* mutation carriers.

Prolonged sedentary behavior has been associated with increased cancer incidence and mortality, independent of physical activity levels, making this an important public health concern (8, 37, 46). Although the deleterious effects of increased sedentary time on cancer risk may be due to reduced physical activity along with the resultant metabolic and hormonal consequences (47), our data suggest that alterations in gene expression may also be involved. To our knowledge, this represents the first evaluation of physical activity/sedentary time and gene expression in women, including those with a hereditary predisposition. It has been suggested that characteristics of sedentary time, including how sedentary time is accrued throughout the day (i.e., long periods/few interruptions vs. short periods/more interruptions), are also important, and that breaking up sedentary time can beneficially affect markers of adiposity, insulin resistance, and inflammation (48–50). Our findings suggest that alterations in gene expression

Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

may also be involved, but the detailed mechanism, likely caused by the interplay of the above, will be the focus of future studies.

Although the mechanism linking prolonged sedentary bouts to lower *BRCA1* expression remains unclear, it may involve BRCA1's role as a metabolic regulator. It has been shown that prolonged sedentary bouts may increase disease risk and that the underlying mechanism may involve lipoprotein lipase (48). During prolonged inactivity, reduced skeletal muscle contractions decrease lipoprotein lipase activity, resulting in reduced clearance of triglycerides and oral glucose from plasma (48). A similar energy balance mechanism may link prolonged sedentary bouts with reduced *BRCA1* expression. BRCA1 is a key regulator of metabolism; by binding to the inactive form of Acetyl-CoA Carboxylase (ACCA), BRCA1 reduces the rate of long-chain fatty acid synthesis (51, 52). Thus, lipoprotein lipase and BRCA1 play complementary roles in response to increased energy needs: lipoprotein lipase breaks down fats and BRCA1 reduces their synthesis.

Strengths associated with the current study include the use of accelerometer-derived data to objectively quantify physical activity levels (53, 54). Although our results provide an exciting direction for prevention research, there are some limitations of our study, including the small sample size, relative homogeneity of our population in terms of physical activity/inactivity, and the quantification of gene expression in peripheral blood lymphocytes rather than the tissue of interest (i.e., breast; refs. 43, 55, 56). More participants representing wider extremes of physical activity and sedentary behavior levels may have yielded larger differences in the relationship with *BRCA1* expression. Furthermore, although physical activity/inactivity differentially affects expression, there are also tissue-specific transcriptional changes that need to be assessed in detail. Although our study did not have sufficient samples to conduct stratified analysis by *BRCA1* mutation status, we did not expect to detect significant differences in *BRCA1* gene expression in women with and without a mutation for various reasons. First, there were five distinct *BRCA1* mutations among the 13 study participants with a known *BRCA1* mutation. Nine of the 13 mutation carriers shared one of two specific mutations, one in exon 20 (5382insC) and the other close to the translation initiation codon in exon 2 (185delAG), both of which are likely to escape nonsense-mediated mRNA decay. Thus, we would not expect to detect lower mRNA levels (57). Although the other four women shared three distinct germline mutations that would result in nonsense-mediated mRNA decay and likely lower expression, the relatively small sample size would likely not result in collectively lower levels compared with noncarriers. Future studies using RNA sequencing, which can discriminate between levels of the wild-type and mutant allele, are warranted. Also, a more comprehensive evaluation of lifestyle needs to be conducted, to ensure we identify all possible associations of (in) activity measures with disease-related gene expression.

In summary, results from this pilot study suggest that prolonged periods of sedentary behavior are inversely associated with *BRCA1* mRNA expression. Whether this association also exists at the protein level, or importantly, translates to enhanced cancer risk remains unknown, but it is suggestive of a possible detrimental role of long periods of uninterrupted sedentary time on *BRCA1* gene expression. Taken together, findings from this study provide important preliminary insight into how sedentary time and physical activity may mediate cancer risk in this high-risk population. This study offers the potential for a clinically important paradigm shift in the prevention strategies available for *BRCA1* mutation carriers. The prospect of changing lifestyle for the purpose of preventing breast cancer in high-risk women, complemented by mechanistic evidence, warrants evaluation in large-scale prospective studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.A. Narod, M. Akbari, L. Salmena, J. Kotsopoulos
Development of methodology: R. Pettapiece-Phillips, L. Salmena, S.A. Narod, M. Akbari, I. Jurisica, J. Kotsopoulos
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Pettapiece-Phillips, R. Chehade, S.A. Narod, M. Akbari, J. Kotsopoulos
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Kotlyar, R. Chehade, L. Salmena, S.A. Narod, M. Akbari, I. Jurisica, J. Kotsopoulos
Writing, review, and/or revision of the manuscript: R. Pettapiece-Phillips, R. Chehade, M. Kotlyar, I. Jurisica, S.A. Narod, M. Akbari, L. Salmena, J. Kotsopoulos
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Pettapiece-Phillips, J. Kotsopoulos
Study supervision: S.A. Narod, M. Akbari, L. Salmena, J. Kotsopoulos

Grant Support

J. Kotsopoulos is the recipient of a Cancer Care Ontario Research Chair in Population Studies. J. Kotsopoulos and M. Akbari are recipients of a Canadian Cancer Society Career Development Award in Prevention. L. Salmena is a recipient of a Tier 2 Canada Research Chair. S.A. Narod and I. Jurisica are recipients of a Tier I Canada Research Chair.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 27, 2015; revised September 25, 2015; accepted October 21, 2015; published OnlineFirst November 2, 2015.

References

1. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117–30.
2. Lubinski J, Huzarski T, Byrski T, Lynch HT, Cybulski C, Ghadirian P, et al. The risk of breast cancer in women with a BRCA1 mutation from North America and Poland. Int J Cancer 2012;131:229–34.
3. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst 2013;105:812–22.
4. Kotsopoulos J, Narod SA. Towards a dietary prevention of hereditary breast cancer. Cancer Causes Control 2005;16:125–38.
5. Friedenreich CM, Cust AE. Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med 2008;42:636–47.
6. Wu Y, Zhang D, Kang S. Physical activity and risk of breast cancer: a meta-analysis of prospective studies. Breast Cancer Res Treat 2013;137:869–82.
7. Lynch BM, Neilson HK, Friedenreich CM. Physical activity and breast cancer prevention. Recent Results Cancer Res 2011;186:13–42.
8. Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. Ann Intern Med 2015;162:123–32.
9. Lynch BM, Healy GN, Dunstan DW, Owen N. Sedentary versus inactive: distinctions for disease prevention. Nat Rev Cardiol 2010;7.

Published OnlineFirst November 2, 2015; DOI: 10.1158/1940-6207.CAPR-15-0291

10. Pettapiece-Phillips R, Narod SA, Kotsopoulos J. The role of body size and physical activity on the risk of breast cancer in BRCA mutation carriers. Cancer Causes Control 2015;26:333–44.
11. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643–6.
12. Kotsopoulos J, Olopado OI, Ghadirian P, Lubinski J, Lynch HT, Isaacs C, et al. Changes in body weight and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2005;7:R833–43.
13. Pijpe A, Manders P, Brohet RM, Collee JM, Verhoef S, Vasen HF, et al. Physical activity and the risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat 2010;120:235–44.
14. Neilson HK, Conroy SM, Friedenreich CM. The influence of energetic factors on biomarkers of postmenopausal breast cancer risk. Curr Nutr Rep 2014;3:22–34.
15. Liu D, Sartor MA, Nader GA, Gutmann L, Treutelaar MK, Pistilli EE, et al. Skeletal muscle gene expression in response to resistance exercise: sex specific regulation. BMC Genomics 2010;11:659.
16. Radom-Aizik S, Zaldivar F Jr, Leu SY, Cooper DM. A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females. J Appl Physiol 2009; 107:168–75.
17. Radom-Aizik S, Zaldivar F Jr., Leu SY, Galassetti P, Cooper DM. Effects of 30 min of aerobic exercise on gene expression in human neutrophils. J Appl Physiol 2008;104:236–43.
18. Catoire M, Mensink M, Boekschoten MV, Hangelbroek R, Muller M, Schrauwen P, et al. Pronounced effects of acute endurance exercise on gene expression in resting and exercising human skeletal muscle. PLoS One 2012;7:e51066.
19. Ronn T, Volkov P, Davegardh C, Dayeh T, Hall E, Olsson AH, et al. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet 2013;9:e1003572.
20. Gjevestad GO, Holven KB, Ulven SM. Effects of exercise on gene expression of inflammatory markers in human peripheral blood cells: a systematic review. Curr Cardiovasc Risk Rep 2015;9:34.
21. Salmena L, Narod S. BRCA1 haploinsufficiency: consequences for breast cancer. Women's Health 2012;8:127–9.
22. Wang M, Yu B, Westerlind K, Strange R, Khan G, Patil D, et al. Prepubertal physical activity up-regulates estrogen receptor beta, BRCA1 and p53 mRNA expression in the rat mammary gland. Breast Cancer Res Treat 2009;115:213–20.
23. Corder K, Brage S, Ekelund U. Accelerometers and pedometers: methodology and clinical application. Curr Opin Clin Nutr Metab Care 2007;10: 597–603.
24. Vanhelst J, Mikulovic J, Bui-Xuan G, Dieu O, Blondeau T, Fardy P, et al. Comparison of two ActiGraph accelerometer generations in the assessment of physical activity in free living conditions. BMC Res Notes 2012;5:187.
25. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc 2008;40:181–8.
26. Swartz AM, Strath SJ, Bassett DR Jr, O'Brien WL, King GA, Ainsworth BE. Estimation of energy expenditure using CSA accelerometers at hip and wrist sites. Med Sci Sports Exerc 2000;32(9 Suppl):S450–6.
27. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32(9 Suppl):S498–504.
28. Bayatti N, Cooper-Knock J, Bury JJ, Wyles M, Heath PR, Kirby J, et al. Comparison of blood RNA extraction methods used for gene expression profiling in amyotrophic lateral sclerosis. PLoS One 2014;9:e87508.
29. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 2008;26:317–25.
30. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 2006;7:3.
31. Fleige S, Pfaffl MW. RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 2006;27:126–39.
32. Nanostring Technologies I. nCounter expression data analysis guide. [cited 2014 May 20]. Available from: http://www.nanostring.com/media/pdf/MAN_nCounter_Gene_Expression_Data_Analysis_Guidelines.pdf%3E, 2012.
33. Rahmati S, Abovsky M, Pastrello C, Chorlton S, Sheahan N, Kotlyar M , et al. pathDIP: A comprehensive resource for known and predicted human gene-pathway associations. (http://ophid.utoronto.ca/navigator/).
34. Kamburov A, Stelzl U, Lehrach H, Herwig R. The ConsensusPathDB interaction database: 2013 update. Nucleic Acids Res 2013;41(Database issue):D793–800.
35. Brown KR, Jurisica I. Unequal evolutionary conservation of human protein interactions in interologous networks. Genome Biol 2007;8:R95.
36. Kotlyar M, Pastrello C, Pivetta F, Lo Sardo A, Cumbaa C, Li H, et al. In silico prediction of physical protein interactions and characterization of interactome orphans. Nat Methods 2015;12:79–84.
37. Shen D, Mao W, Liu T, Lin Q, Lu X, Wang Q, et al. Sedentary behavior and incident cancer: a meta-analysis of prospective studies. PLoS One 2014;9: e105709.
38. Schmid D, Leitzmann MF. Television viewing and time spent sedentary in relation to cancer risk: a meta-analysis. J Natl Cancer Inst 2014;106.
39. Janssen I. Health care costs of physical inactivity in Canadian adults. Appl Physiol Nutr Metab 2012;37:803–6.
40. World Health Organization. Global Strategy on Diet, Physical Activity and Health. Physical Activity and Adults. Available from: http://www.who.int/dietphysicalactivity/factsheet_adults/en/.
41. Huen MS, Sy SM, Chen J. BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol 2010;11:138–48.
42. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 2004;4:665–76.
43. Kotsopoulos J, Zhang S, Akbari M, Salmena L, Llacuachaqui M, Zeligs M, et al. BRCA1 mRNA levels following a 4-6-week intervention with oral 3,3′-diindolylmethane. Br J Cancer 2014;111:1269–74.
44. Magbanua MJ, Richman EL, Sosa EV, Jones LW, Simko J, Shinohara K, et al. Physical activity and prostate gene expression in men with low-risk prostate cancer. Cancer Causes Control 2014;25:515–23.
45. Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P. Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 2006;98:285–94.
46. Kohl HW 3rd, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, et al. The pandemic of physical inactivity: global action for public health. Lancet 2012;380:294–305.
47. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579–91.
48. Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, et al. Breaks in sedentary time: beneficial associations with metabolic risk. Diabetes Care 2008;31:661–6.
49. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. Eur Heart J 2011;32:590–7.
50. Lynch BM, Friedenreich CM, Winkler EA, Healy GN, Vallance JK, Eakin EG, et al. Associations of objectively assessed physical activity and sedentary time with biomarkers of breast cancer risk in postmenopausal women: findings from NHANES (2003–2006). Breast Cancer Res Treat 2011;130:183–94.
51. Magnard C, Bachelier R, Vincent A, Jaquinod M, Kieffer S, Lenoir GM, et al. BRCA1 interacts with acetyl-CoA carboxylase through its tandem of BRCT domains. Oncogene 2002;21:6729–39.
52. Jackson KC, Gidlund EK, Norrbom J, Valencia AP, Thomson DM, Schuh RA, et al. BRCA1 is a novel regulator of metabolic function in skeletal muscle. J Lipid Res 2014;55:668–80.
53. Shephard RJ. Limits to the measurement of habitual physical activity by questionnaires. Br J Sports Med 2003;37:197–206.
54. Prince SA, Adamo KB, Hamel ME, Hardt J, Connor Gorber S, Tremblay M. A comparison of direct versus self-report measures for assessing physical activity in adults: a systematic review. Int J Behav Nutr Phys Act 2008;5:56.
55. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, et al. Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci U S A 2003;100:1896–901.
56. Cabanes A, Wang M, Olivo S, DeAssis S, Gustafsson JA, Khan G, et al. Prepubertal estradiol and genistein exposures up-regulate BRCA1 mRNA and reduce mammary tumorigenesis. Carcinogenesis 2004;25:741–8.
57. Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM, Mazoyer S. The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. Hum Mol Genet 2002;11:2805–14.

AACR American Association for Cancer Research

Cancer Prevention Research

Uninterrupted Sedentary Behavior Downregulates *BRCA1* Gene Expression

Rachael Pettapiece-Phillips, Max Kotlyar, Rania Chehade, et al.

Cancer Prev Res 2016;9:83-88. Published OnlineFirst November 2, 2015.