



## The effect of prescribed exercise volume on biomarkers of chronic stress in postmenopausal women: Results from the Breast Cancer and Exercise Trial in Alberta (BETA)

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### ABSTRACT

There is epidemiologic and biologic evidence for a role of stress in breast cancer etiology and physical activity mitigates the negative effects of stress. We examined the potential for a dose-response relationship between two volumes of aerobic exercise and biomarkers of chronic stress in post-menopausal women. The Breast Cancer and Exercise Trial in Alberta is a randomized controlled trial with post-menopausal women randomized to either a MODERATE (150 min per week) or HIGH (300 min per week) volume of exercise over a one year intervention period. Fasting serum concentrations of cortisol, cortisone, corticosterone and 11-deoxycortisol at baseline, 12 months (the end of the intervention), and 24 months. Intention-to-treat analyses were performed using general linear models, adjusted for baseline biomarker concentrations. There were modest but non-statistically significant decreases in cortisol (HIGH: -4%, 95% CI: -7%, 2%; MODERATE: -1%, 95% CI: -14%, 4%) and corticosterone (HIGH: -4%, 95% CI: -12%, 6%; MODERATE: -5%, 95% CI: -14%, 4%) concentrations for both exercise groups between baseline and 12 months, and no difference in cortisone concentrations. Intention-to-treat analysis of 386 (97%) participants showed no statistically significant group differences for changes in biomarker levels at 12 months. Between baseline and 12 months, there were no differences in cortisol or cortisone and, at 24 months all stress hormone levels increased to near-baseline levels with no significant differences between the two intervention groups.

### 1. Introduction

There is accumulating evidence that increased psychosocial stress is associated with increased cancer risk (Chida et al., 2008). Epidemiologic and biologic evidence exists to support a role for stress in breast cancer etiology (Antonova et al., 2011). Stressful early life events change the long-term function and stress reactivity of the hypothalamic-pituitary-adrenal (HPA) axis, which has been associated with increased cancer risk (Antonova et al., 2011). Stress activates the HPA axis, which then releases glucocorticoid hormones. Glucocorticoids are responsible for a variety of physiological processes including metabolism, cell growth, apoptosis, and immune response (Sorensen et al., 2012). Response of these hormones is mediated by glucocorticoid receptors (GRs), which are heavily present in both healthy and cancerous

breast cells and promote mammary cell proliferation and apoptosis (Pudrovska et al., 2013). Due to this physiological involvement, it is feasible that glucocorticoids and stress play a role in breast cancer development.

Serum cortisol, corticosterone, cortisone, and 11-deoxycortisol are key biomarkers for stress response. Cortisol is perhaps the most well-known glucocorticoid in the human body and has an established relationship with acute stress (Adam et al., 2006; Sephton and Spiegel, 2003). Corticosterone is another important corticoid, and can reflect recent stress over the interval of hours to days, which is between traditional definitions of acute and chronic stress (Hewitt et al., 2014; Koren et al., 2012; McCorkell et al., 2013). Concentrations of cortisone and 11-deoxycortisol have been explored as the substrate pool for cortisol synthesis and the first cortisol clearance product (Tomlinson

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et al., 2004). Concentrations of these biomarkers are typically far lower than for cortisol but may also be indicators of acute stress. These biomarkers of chronic stress can then be measured in serum, saliva, and hair.

Physical activity is associated with reduced risk of breast cancer through several mechanisms, including by regulating sex hormones, maintaining a healthy weight, reducing inflammation, and improving the immune response (de Boer et al., 2017). Physical activity mitigates the negative effects of stress in older adults (Heaney et al., 2014), buffers the impact of chronic stress on telomere shortening (Puterman et al., 2010), desensitizes tissue sensitivity to prolonged cortisol exposure (Duclos et al., 2003), reduces cortisol responses to acute stressors (Zschucke et al., 2015), and decreases elevated morning cortisol in older adults (Mura et al., 2014). Several cross-sectional studies have demonstrated statistically significant decreased levels of cortisol (serum, urinary or salivary) in those who are physically active compared to those who are sedentary (Lai et al., 2017; Sotos-Prieto et al., 2015; Zawadzki et al., 2015). Beneficial effects on cortisol levels have also been demonstrated in physical activity intervention studies (Arazi et al., 2013; Roberts et al., 2013). However, an important question that remains unanswered is the appropriate dose of physical activity required to receive a beneficial effect on stress hormones. In addition, few studies have been conducted in postmenopausal women.

The present study aims to assess the effect of physical activity on concentrations of stress biomarkers at different doses of exposure. The Breast Cancer and Exercise Trial in Alberta (BETA) was a randomized controlled trial (RCT) that studied the effects of a physical activity intervention on biomarkers of breast cancer risk in previously inactive, postmenopausal women who are particularly at risk for breast cancer and who could benefit from a physical activity intervention. Women were randomized to either a MODERATE (150 min/week) or HIGH (300 min/week) volume of moderate aerobic exercise for one year. In this ancillary analysis, we investigated the dose-response effects of a year-long aerobic exercise intervention on blood biomarkers of stress, specifically serum cortisol, cortisone, corticosterone and 11-deoxycortisol in postmenopausal women.

## 2. Methods

### 2.1. Study population

Study methods for BETA have previously been described in detail (Friedenreich et al., 2014). Briefly, BETA was a two-armed, two-centered (Calgary and Edmonton, Alberta) randomized controlled trial that included 400 postmenopausal, previously inactive women and was conducted between July 2010 and April 2013. The trial consisted of a 12-month exercise intervention and a 24-month follow-up after intervention completion. The study protocol was approved by the Alberta Cancer Research Ethics Committee, the Conjoint Health Research Ethics Board of the University of Calgary and the Health Research Ethics Board of the University of Alberta. All participants provided signed informed consent to participate prior to enrolment.

Eligibility criteria for participation in BETA included: 1) resident of Calgary or Edmonton; 2) 50–74 years of age; 3) non-hormone therapy user within the 12 months prior to enrolment; 4) body mass index (BMI) between 22 and 40 kg/m<sup>2</sup>; 5) < 14 alcoholic drinks per week on average; 6) non-smoker; 7) inactive (defined below); 8) able to do unrestricted or progressive physical activity as assessed by physician screening; 9) normal levels of cholesterol, fasting blood glucose (< 7 mmol/L), thyroid stimulating hormone and alanine aminotransferase; 10) cancer-free; and 11) not on a weight loss program or planning to commence one. Postmenopausal status was defined as one of: natural cessation of menstrual periods for at least 24 months, bilateral oophorectomy, hysterectomy without bilateral oophorectomy and age ≥ 55 years, or hysterectomy without bilateral oophorectomy and age 50–54 years with a follicle-stimulating hormone

level > 30 IU/L. Inactivity was defined as < 90 min/week of exercise or if between 90 and 120 min/week, having a VO<sub>2max</sub> < 34 mL/kg/min as assessed by a sub-maximal fitness test.

### 2.2. Exercise intervention

Women were randomly allocated into either a MODERATE (150 min per week) or HIGH (300 min per week) volume of exercise per week. All participants were prescribed the same intensity of physical activity (target of 70–80% heart rate reserve) and exercised five times per week. Three days per week were supervised by certified exercise trainers, while two days per week consisted of home-based unsupervised sessions. Adherence to the exercise intervention was monitored using weekly exercise logs and heart rate monitors were worn during each session to ensure at least 50% of the exercise time in each session was completed within the target heartrate zone. Participants were requested not to change their normal dietary intake.

### 2.3. Biomarker assays

Fasting blood samples were collected from each participant at baseline, 6, 12 and 24 months. Blood draws were performed following a minimum 10-h fast and complete abstinence from exercise and alcohol for 24 h. Serum aliquots were stored within 12 h of collection in a – 86 °C freezer until the time of assay. Fasting serum concentrations of cortisol, cortisone, corticosterone and 11-deoxycortisol were measured at baseline, 12-months, and at 24-months (one year following the exercise intervention). Serum samples were batched such that each batch contained baseline and follow-up samples for the same woman, and an equal number of both intervention arm samples. Pooled quality assurance samples were included in each batch to estimate intra- and inter-assay coefficients of variation (CV).

Cortisol, cortisone, corticosterone, and 11-deoxycortisol were purchased from Steraloids (Newport, RI). Deuterium labeled internal standards (Cortisol-d4 and corticosterone-d8) were obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada). Serum sample preparation by protein precipitation (cold ZnSO<sub>4</sub>·7H<sub>2</sub>O solution, 9 mg/mL) introduced the internal standard. Samples were separated on an Agilent 1200 binary liquid chromatography (LC) system connected with an AB SCIEX QTRAP® 5500 tandem mass spectrometer equipped with electrospray ionization (ESI) source. LC separation was performed on a ZORBAX Eclipse plus C18 column (100 × 2.1 mm, 1.8 μm particle size) at 40 °C. Analytes, including deuterated internal standards, were detected in positive mode with qualifier and a quantifier transitions (Q1 and Q3) as follows: cortisol (363/121, 363/327), cortisol-d4 (367/121, 367/331), cortisone (361/163, 361/121), corticosterone (347/329, 347/121), corticosterone-d8 (355/337, 355/125), 11-deoxycortisol (347/97, 347/109). Intra- and inter-assay CVs for cortisol, cortisone, corticosterone and 11-deoxycortisol were 3% and 3%, 7% and 8%, 12% and 14%, and 14% and 21%, respectively.

### 2.4. Statistical analysis

Intention-to-treat (ITT) analyses using linear models, adjusted for baseline biomarker concentrations, were performed to examine intervention effects from baseline to 12-months and from baseline to 24-months. Mean values were calculated for the difference at each intervention level and for each hormone. Between-group differences in means were then estimated to determine the effect of the intervention for each hormone. Per-protocol analysis was also performed to assess the effect of non-compliance on effect estimates. Inclusion in this sample was defined as 90% adherent to intervention, which translated to at least 270 min per week for the HIGH volume group, and 135–150 min per week for the MODERATE volume group.

A secondary analysis examining long-term intervention effects from baseline to 24-months was also performed using linear models, adjusted

for baseline biomarker concentrations, similar to the primary ITT analysis. Potential moderation (effect modification) of the intervention effect was hypothesized *a priori* and evaluated using the value of the interaction term ( $P_{\text{heterogeneity}}$ ) between the randomization group assignment and each proposed moderator at baseline in the models used in the ITT analysis with the addition of the proposed moderator. Hypothesized moderators of the present analysis were continuous variables and included baseline: age, BMI, study site, physical fitness ( $\text{VO}_{2\text{max}}$ ), measures related to stress, such as quality of life (36-Item Short Form Survey – SF-36) and sleep quality (Pittsburgh Sleep Quality Index) (Buysse et al., 1989; Ware Jr. and Sherbourne, 1992). For those interactions that were deemed to be statistically significant ( $p < 0.05$ ), subgroup analyses were performed by dividing the population at the median for each variable, with the exception of BMI, which was grouped by the clinically relevant groups of normal weight/overweight ( $\text{BMI} < 30 \text{ kg/m}^2$ ) and obese ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ). As we did not mandate a specific time for biomarker collection, sensitivity analyses were performed on a time of day variable for biomarker sample collection to test the potential impact of morning cortisol sampling on results. Included in the sensitivity analysis were all participants who had blood collections prior to 9 AM at baseline, 12-months and 24-months. All statistical analyses were performed using SAS software (Version 9.3; SAS Institute).

### 3. Results

A study flow diagram of participants through BETA has previously been detailed elsewhere (Friedenreich et al., 2015). There was 97% retention of study participants during this trial, thus 195 HIGH volume and 191 MODERATE participants were included in the analysis of the baseline and 12-months data. Of these 386 participants who completed the trial, there were 333 who returned for the 24-month assessments, with 168 and 165 participants in the HIGH and MODERATE volume groups, respectively. Reasons for losses-to-follow-up included inability to contact, refusal, medical reasons/death, moving or incomplete follow-up questionnaire data.

Concentrations of stress biomarkers fell within the normal reference range for both the MODERATE and HIGH volume exercise groups

**Table 1**

Baseline characteristics of randomized participants in the Breast Cancer and Exercise Trial in Alberta (BETA), 2010–2013.

Baseline characteristic	MODERATE volume exercise ( $n = 191$ ) Mean (SD)	HIGH volume exercise ( $n = 195$ ) Mean (SD)
Age (years)	59.6 (5.1)	59.4 (4.9)
$\text{VO}_{2\text{max}}$ (mL/kg/min)	26.8 (4.9)	26.8 (5.2)
Body mass index ( $\text{kg/m}^2$ )	29.5 (4.5)	29.0 (4.4)
Biomarkers	Median (IQR)	Median (IQR)
Cortisol (nmol/L)	367 (273–450)	386 (301–477)
Cortisone (nmol/L)	62.7 (48.8–76.8)	64.4 (49.9–79.6)
Corticosterone <sup>a</sup> (nmol/L)	7.8 (4.9–12.7)	10.1 (5.8–14.4)
Other characteristics	Mean (SD)	Mean (SD)
Global sleep quality	6.0 (3.4)	6.0 (3.6)
General health	53.8 (6.7)	64.4 (49.9–79.6)

Abbreviations: SD, standard deviation;  $\text{VO}_{2\text{max}}$ , maximal oxygen consumption; IQR, interquartile range.

Global sleep quality: Measured by the Pittsburgh Sleep Quality Index and provides a score between 0 and 21. Higher scores indicate worse sleep quality. General health: A component of the Medical Outcomes Study Short Form-36 Survey (SF-36) with scores ranging from 0 to 100. Higher scores indicate higher function or well-being.

<sup>a</sup> There were no statistically significant differences at baseline between HIGH and MODERATE groups for these variables, with the exception of corticosterone ( $p = 0.02$ ) and 11-deoxycortisol ( $p = 0.04$ ), using the Wilcoxon rank sum test.

(Table 1) (Tavita and Greaves, 2017). Median levels for steroid levels in the MODERATE and HIGH volume groups are provided in Table 1. There were no differences in any baseline characteristics, including biomarker concentrations, with the exception of corticosterone ( $p = 0.02$ ) and 11-deoxycortisol ( $p = 0.04$ ). These values were slightly higher in the HIGH volume exercise group.

Our primary ITT analysis of changes in chronic stress biomarker concentrations between baseline and 12-months did not reveal any statistically significant differences between intervention groups for any biomarker (Table 2). There were modest reductions in concentrations of cortisol (HIGH:  $-7.62 \text{ nmol/L\%}$ , 95% CI:  $-26, 10.74$ ; MODERATE:  $-10.3 \text{ nmol/L\%}$ , 95% CI:  $-28.9, 8.25$ ) for both interventions groups between baseline and 12-months, but these changes were not statistically significant. In our secondary analysis of long-term intervention effects examining changes in chronic stress biomarkers between baseline and 24-months (12-months following the end of the exercise intervention), we also did not observe any statistically significant differences in changes between intervention groups (Table 3). Lastly, in interaction tests for potential effect modification, baseline BMI was found to have a statistically significant interaction and there were no significant findings for the other interaction terms (Table 4). The subgroup analysis revealed that those with  $\text{BMI} < 30$  in the HIGH volume group had a significantly larger decrease in the cortisol:corticosterone ratio compared to the MODERATE volume group (TER = 0.93, 95% CI: 0.88–0.99). No other statistically significant effects were observed for the other chronic stress biomarkers across subgroups of BMI.

The per-protocol analysis was conducted to determine the impact of intervention compliance on effect estimates. The sample consisted of 138 women who were  $\geq 90\%$  adherent to intervention group (80 in HIGH volume group, 58 in MODERATE volume group). No statistically significant associations were found for any of the steroid biomarkers (Supplementary Tables 1 and 2).

### 4. Discussion

The results of our analysis did not reveal any statistically significant effects of a HIGH versus MODERATE volume aerobic exercise intervention on biomarkers of chronic stress in postmenopausal women, with the exception of those with  $\text{BMI} < 30 \text{ kg/m}^2$ , where the cortisol:corticosterone ratio was found to be significantly decreased in the HIGH volume exercise group compared to the MODERATE volume exercise group. Higher levels of cortisol may be associated with higher BMI levels as we also found a statistically significant interaction when BMI was included in the model. Cortisol is associated with the acute stress response in humans, and cortisol concentrations tend to be considerably higher than corticosterone (cortisol dominance). However, there is evidence that the non-dominant glucocorticoid (corticosterone in humans) does not simply track the dominant, but can have a different temporal pattern of activation by stressors (Hewitt et al., 2014; Koren et al., 2012; Wynne-Edwards et al., 2013). Corticosterone is traditionally viewed as a secondary response following initial increases in cortisol levels. Decreases in the cortisol:corticosterone ratio generally reflect an increase in corticosterone, suggesting long-term stress. Our study results indicate that women with a  $\text{BMI} < 30 \text{ kg/m}^2$  may have experienced this long-term stress in the HIGH exercise group compared to the MODERATE exercise group, suggesting that the HIGH volume of exercise may have caused low levels of long-term stress. However, this interpretation should be read with caution because the analyses of hormone ratios here are exploratory in nature.

Although the epidemiologic literature is inconsistent, there is accumulating evidence that physical activity can decrease levels of stress biomarkers, specifically cortisol (Arazi et al., 2013; Lai et al., 2017; Roberts et al., 2013; Sotos-Prieto et al., 2015; Zawadzki et al., 2015), across different healthy study populations (Hayes et al., 2015; Laugero et al., 2011; Vaamonde et al., 2012; von Kanel et al., 2017). Conversely, several studies of physical activity interventions did not have any effect

**Table 2**

Intention-to-treat analysis of chronic stress biomarkers for HIGH and MODERATE volume exercise groups in the Breast Cancer and Exercise trial in Alberta (2010–2013) at baseline and 12-months.

Biomarkers	N <sup>a</sup>	Baseline Mean (95% CI)	12 months Mean (95% CI)	Mean change (95% CI)	Difference in mean change (95% CI)	P-value
Cortisol (nmol/L)						
HIGH	195	399.1 (379.0, 419.3)	384.1(363.2, 405.0)	−7.62 (−26, 10.74)	2.69 (−23.5, 28.83)	0.84
MODERATE	191	378.1 (358.4, 397.9)	375.4 (357.9, 392.9)	−10.3 (−28.9, 8.25)		
Cortisone (nmol/L)						
HIGH	195	65.67 (62.78, 68.57)	65.83 (62.62, 69.04)	0.45 (−2.42, 3.31)	0.61 (−3.46, 4.68)	0.77
MODERATE	191	64.58 (61.51, 67.66)	64.72 (61.53, 67.90)	−0.16 (−3.06, 2.73)		
Corticosterone (nmol/L)						
HIGH	195	11.53 (10.37, 12.69)	11.48 (9.96, 13.01)	0.48 (−0.73, 1.70)	1.12 (−0.61, 2.85)	0.20
MODERATE	191	9.68 (8.72, 10.63)	9.58 (8.58, 10.58)	−0.64 (−1.86, 0.59)		
11-deoxycortisol (nmol/L)						
HIGH	194	0.95 (0.86, 1.05)	0.91 (0.82, 1.01)	0.0 (−0.08, 0.08)	0.05 (−0.06, 0.17)	0.37
MODERATE	191	0.82 (0.74, 0.91)	0.81 (0.73, 0.89)	−0.05 (−0.14, 0.03)		
Cortisol:cortisone						
HIGH	195	6.25 (5.98, 6.51)	6.01 (5.74, 6.27)	−0.19 (−0.40, 0.03)	−0.19 (−0.50, 0.12)	0.23
MODERATE	191	6.00 (5.77, 6.23)	6.06 (5.80, 6.31)	0.0 (−0.22, 0.22)		
Cortisol:corticosterone						
HIGH	195	46.45 (42.25, 50.65)	48.01 (44.26, 51.75)	0.64 (−2.17, 3.46)	−0.72 (−4.73, 3.29)	0.72
MODERATE	191	50.10 (46.68, 53.52)	50.54 (47.51, 53.57)	1.37 (−1.48, 4.22)		
Cortisol:11-deoxycortisol						
HIGH	194	525.5 (490.4, 560.7)	535.4 (497.2, 573.5)	1.89 (−30.00, 33.74)	−26.2 (−71.4, 19.14)	0.26
MODERATE	191	564.3 (536.7, 601.9)	584.3 (545.5, 623.0)	28.04 (−4.06, 60.15)		

<sup>a</sup> One sample contained invalid measures for 11-deoxycortisol at 12 months due to laboratory error and was excluded from analysis.

**Table 3**

Analysis of chronic stress biomarkers for HIGH and MODERATE volume exercise groups in the Breast Cancer and Exercise Trial in Alberta (2010–2013) at baseline and 24-months.

Biomarker	N <sup>a</sup>	Baseline Mean (95% CI)	24 months Mean (95% CI)	Mean change (95% CI)	Difference in mean change (95% CI)	P-value
Cortisol (nmol/L)						
HIGH	168	394.1 (372.5, 415.7)	410.6 (388.5, 432.7)	19.62 (−0.73, 39.97)	−1.64 (−30.60, 27.32)	0.91
MODERATE	164	384.0 (362.4, 405.6)	408.5 (386.6, 430.4)	21.26 (0.67, 41.86)		
Cortisone (nmol/L)						
HIGH	168	65.26 (62.09, 68.43)	68.32 (65.25, 71.38)	2.98 (0.07, 5.88)	1.54 (−5.67, 2.59)	0.46
MODERATE	164	65.51 (62.16, 68.85)	69.94 (66.75, 73.13)	4.52 (1.58, 7.45)		
Corticosterone (nmol/L)						
HIGH	168	11.45 (10.19, 12.70)	12.33 (10.67, 13.98)	1.24 (−0.15, 2.62)	−0.28 (−2.25, 1.69)	0.78
MODERATE	164	9.84 (8.79, 10.88)	11.71 (10.33, 13.10)	1.52 (0.12, 2.91)		
11-deoxycortisol (nmol/L)						
HIGH	168	0.95 (0.84, 1.05)	1.01 (0.89, 1.14)	0.09 (−0.01, 0.19)	0.01 (−0.13, 0.15)	0.91
MODERATE	165	0.83 (0.74, 0.92)	0.94 (0.83, 1.04)	0.08 (−0.02, 0.18)		
Cortisol:cortisone						
HIGH	168	6.22 (5.92, 6.52)	6.20 (5.89, 6.52)	0.02 (−0.23, 0.26)	0.0 (−0.35, 0.35)	1.00
MODERATE	164	5.99 (5.74, 6.23)	6.04 (5.73, 6.35)	0.02 (−0.23, 0.27)		
Cortisol:corticosterone						
HIGH	168	45.84 (41.55, 50.12)	46.50 (42.87, 50.14)	−0.52 (−3.71, 2.67)	1.56 (−2.99, 6.10)	0.50
MODERATE	164	49.52 (46.19, 52.85)	46.23 (42.92, 49.53)	−2.08 (−5.31, 1.15)		
Cortisol:11-deoxycortisol						
HIGH	168	523.4 (485.6, 561.2)	547.4 (505.6, 589.3)	16.97 (−14.00, 47.99)	25.66 (−18.50, 69.87)	0.25
MODERATE	164	563.9 (525.4, 602.4)	548 (510.4, 585.5)	−8.69 (−40.10, 22.71)		

<sup>a</sup> One sample contained invalid measures for 11-deoxycortisol at 12 months due to laboratory error and was excluded from analysis.

on resting cortisol levels measured in urine, saliva or blood (Arikawa et al., 2013; Brumby et al., 2013; Grandys et al., 2009; Hiruntrakul et al., 2010; Prick et al., 2015; Vaczi et al., 2014; Vale et al., 2009). One physical activity intervention trial in postmenopausal women demonstrated beneficial statistically significant changes in tumour necrosis factor- $\alpha$  and the cortisol to dehydroepiandrosterone sulfate ratio, but no changes in cortisol (Izzicupo et al., 2013). The present study also did not find a statistically significant change in cortisol levels in response to exercise in either intervention group.

It is worthwhile to note the limitations of this study. First, hormone measures were taken at multiple time-points throughout the day and we did not record the time of waking. As noted earlier, cortisol levels tend to be substantially higher upon wakening and decrease sharply with wakefulness (Adam et al., 2006), which may lead to measurement

error. However, sensitivity analysis revealed that time of the morning cortisol collection had no impact on final results (Supplementary Table 1). The majority of biomarker collection occurred between 07:30 and 11:00 and when the analysis was restricted to those samples taken between 07:00 and 09:00 there was no significant change in the results. This result is likely due to the quick drop-off in cortisol levels following the spike at waking. Second, the present study used blood biomarkers of cortisol, which may not capture chronic stress as well as other measures, such as hair (Wester and van Rossum, 2015). The use of blood rather than other biologic measurements may have limited the ability to assess circulating cortisol levels over the long-term. Third, the homogenous nature of the study population may make these results less generalizable to a different population. Socioeconomic status of participants was relatively high, which may have an impact on baseline

**Table 4**

Exercise intervention effects on chronic stress biomarkers from baseline to 12-months, stratified by baseline BMI in the Breast Cancer and Exercise Trial in Alberta (2010–2013).

Biomarker	BMI < 30 kg/m <sup>2</sup>		BMI ≥ 30 kg/m	
	n <sup>a</sup>	TER <sup>b</sup>	n <sup>a</sup>	TER
Cortisol	121, 116	0.94 (0.86, 1.02) P <sup>c</sup> = 0.16	74, 75	1.06 (0.94, 1.18) P <sup>c</sup> = 0.35
Cortisone	121, 116	1.00 (0.93, 1.08) P <sup>c</sup> = 0.98	74, 75	1.00 (0.90, 1.12) P <sup>c</sup> = 0.94
Corticosterone	121, 116	0.93 (0.79, 1.10) P <sup>c</sup> = 0.39	74, 75	1.17 (0.94, 1.45) P <sup>c</sup> = 0.17
11-Deoxycortisol	120, 116	0.96 (0.84, 1.10) P <sup>c</sup> = 0.55	74, 75	1.17 (0.98, 1.41) P <sup>c</sup> = 0.09
Cortisol:Cortisone ratio	121, 116	0.93 (0.88, 0.99) P <sup>c</sup> = 0.02	74, 75	1.04 (0.96, 1.13) P <sup>c</sup> = 0.36
Cortisol:Corticosterone ratio	121, 116	1.01 (0.92, 1.10) P <sup>c</sup> = 0.87	74, 75	0.93 (0.82, 1.05) P <sup>c</sup> = 0.23
Cortisol:11-deoxycortisol ratio	121, 116	0.97 (0.89, 1.07) P <sup>c</sup> = 0.56	74, 75	0.92 (0.82, 1.05) P <sup>c</sup> = 0.22

<sup>a</sup> Numbers represent those in the high exercise group and those in the moderate exercise group. HIGH, MODERATE, respectively.

<sup>b</sup> Mean difference calculated as: HIGH-MODERATE group changes from baseline to 12 months.

<sup>c</sup> P-value tests the null hypothesis of no difference in changes between HIGH and MODERATE groups against the two-sided alternative hypothesis.

levels of stress hormones, although this relationship is not clear in the literature (Desantis et al., 2015; Karlamangla et al., 2013; Zilioli et al., 2015). Additionally, there are limitations of the study itself that may have limited our ability to observe a statistically significant result. The first is the sample size of the population, which may limit the opportunity for sufficient power in subgroup analyses and the possibility of a chance finding given the multiple comparisons that were made. It is also possible that the intervention was not done over an appropriate time period to see changes in stress hormones. In addition, other factors might influence levels of glucocorticoids in blood such as dietary and caffeine intakes and stressful life events. While we did examine these factors and did not find them to be effect modifiers of these associations, it is possible that there were other unmeasured factors that we could not consider that might also influence these chronic stress biomarker levels. Finally, in the per-protocol analysis that included all women who adhered to 90% or more of the exercise intervention sessions, there were no differences in these chronic stress biomarker levels suggesting that the intervention did not have any meaningful impact on these biomarkers. The per-protocol analysis was limited to a subset of the study population ( $n = 138$ ) which decreased the study power of this component of the analysis.

While we did not observe any statistically significant differences in overall stress biomarker concentrations, this study fills an important gap in the literature. Few studies have been conducted that examine changes in the metabolic pathway of cortisol, and its precursors and metabolites, during a year-long exercise intervention and observational period. While both groups showed a minor reduction in stress hormone levels during the intervention period, levels subsequently increased in the 12 months of follow-up. This increase in hormone levels following the intervention could be related to other changes and lifestyle factors that occurred during this period. As previously described, both groups self-reported ~180 min/week of moderate-vigorous physical activity between 12- and 24-months. While there were no differences in total body fat at 24-months between the two groups, there were statistically significant differences for body mass index, waist-to-hip ratio and subcutaneous fat area with greater decreases found in the HIGH volume arm (Friedenreich et al., 2019). This finding suggests that body composition has a stronger influence on measures of chronic stress hormones than volume of physical activity.

Future studies should use a larger sample size, which may show more significant differences between groups and should include measurements of waking time as well as blood collection time in order to control for the circadian rhythm influence on cortisol levels. The

alteration in the cortisol:corticosterone ratio in women with a BMI < 30 kg/m<sup>2</sup> in our study population suggests the possibility of an alteration in the adrenal steroid biosynthesis pathway as a result of an exercise intervention. Additionally, cortisone and 11-deoxycortisol have not been traditionally studied as part of the human stress response to physical activity, so an exploratory analysis of these hormones is included in this study and further studies should also include a wide range of biomarkers. To understand the relationship between physical activity, stress, and breast cancer more completely, future investigations should be conducted on the dynamic changes in the biomarkers that make up the human stress response.

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## Authors' contributions

Study design and funding: CMF, KSC, KWE. Study conduct: CMF, KSC, KWE, RZ, DRB, QW. Data analysis and table creation: QW. Manuscript preparation and writing: ES, EH, CMF. Manuscript review and approval: All authors.

## Availability of data and material

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

## Declaration of Competing Interest

No potential conflicts of interest declared.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pmedr.2019.100960>.

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