INTRODUCTION

Given the growing cohort of breast cancer survivors resulting from a combination of early detection and better survival outcomes,1,2 coupled with ongoing psychosocial issues for many including fears of disease recurrence, depression, anxiety, and fatigue,3 interventions to support the survivorship phase have increased in importance and urgency.2 We recently reported primary outcomes of the MINDSET trial, which compared 2 empirically supported psychosocial group interventions, mindfulness-based cancer recovery (MBCR) and supportive-expressive group therapy (SET), with a minimal-intervention control condition on mood, stress symptoms, quality of life, social support, and diurnal salivary cortisol in distressed breast cancer survivors.4 Although MBCR participation resulted in the most psychosocial benefit, including improvements across a range of psychosocial outcomes, both MBCR and SET resulted in healthier cortisol profiles over time compared with the control condition.

In this secondary analysis of MINDSET trial data, we collected and stored blood samples taken from a subset of women to further investigate the effects of these interventions on potentially important biomarkers. Telomeres are specialized nucleoprotein complexes that form the protective ends of linear chromosomes5 and provide genomic stability...
through several mechanisms. Telomere dysfunction and th
loss of telomere integrity may result in DNA damage ou
or cell death; when a critically short telomere length (TL)
is reached, cells enter senescence and have reduced viabili
and chromosomal fusions appear.6 Shorter TL has be
implicated in several disease states, including cardiovascula
de disease, diabetes, dyskeratosis congenita, aplastic a
emia, and idiopathic pulmonary fibrosis.7 Shorter TL al
was found to be predictive of earlier mortality in pa
tients with chronic lymphocytic leukemia,8 promyelo
cytic leukemia,9 and breast cancer.10-12 However, the r
relationships between TL and the clinical or pathological fea
tures of tumors are still not clearly understood.13

Recent emerging research has suggested that TL and its
enzyme telomerase may be susceptible to psychosocial in
fluences, particularly stress.14 Telomerase is the speciali
zed cellular reverse transcriptase that elongates telomeric D
a, thereby counteracting the telomere shortening that oc
urs with successive rounds of cell division.5 The earliest s
udies demonstrated associations between naturally occ
uring stressors and telomere biology in noncancer sampl
s, in which stress was associated with shorter TL and l
ower telomerase activity.15,16

Although stress plays a role in the etiology and progr
ression of many diseases,17-19 the role of stress in cance
remains suggestive.20,21 Nonetheless, several biobehaviou
ral pathways between psychosocial stress and mechanism
s of cancer development have been indentifed.20,22 Telom
ere biology represents another provocative potential pa
thway that may link psychosocial influences with cancer pr
gression.23 One report of associations between decreas
in distress levels and increased TL over a 4-
month period in survivors of cervical cancer is to our kn
owledge the only current evidence of associations betw
een TL and stress in individuals with cancer.23

Intervention studies have now examined the effects of psy
chosocial programs on telomerase activity and TL. An un
controlled study by Ornish et al reported lifestyle cha
ges including a low-fat diet, exercise, and stress reduc
tion in patients with low-risk prostate cancer were si
gnificantly associated with increases in telomerase activi
ty, which was significantly associated with decreases in lo
density lipoprotein, cholesterol, and psychological dis	ress.24 However, there was no control group and TL w
as not measured. More recently, 3 small trials of medita
tion intervention studies measured telomerase activity in
healthy volunteers,25 individuals with obesity,26 and care
givers of patients with Alzheimer disease.27 One study a
lso measured TL in healthy meditators.28 Although each o
of these trials demonstrated promising results, they ha
small sample sizes (ranging from 37-60 participants) an
only postassessment measurements,25 and the one study t
that measured TL (as opposed to telomerase)28 lacked ra
domization. Assessing TL before and after psychoso
cial interventions would allow for the development of an u
derstanding of the potential short-term effects of psy
chosocial support and mind-body practices on TL. We e
pected different results in TL between MBCR and SET. T
his is because TL is susceptible to psychological influen
ces14 and MBCR was found to be superior to SET in i
proving mood and stress symptoms in our previous s
udy.4

The objectives of the current study were to: 1) compa
re the effects of 2 psychosocial interventions for dis	ressed breast cancer survivors with a control condition o
TL, and 2) assess the relationships between changes in TL
and changes in stress and mood. Because the TL data wer
available for only a subset of participants, the current s
udy aimed to demonstrate preliminary evidence of the e
fect of psychooncological interventions on TL so that f
uture research may target TL as a primary outcome with g
reater sample sizes.

MATERIALS AND METHODS

Study Design

The current study used a longitudinal randomized con
trolled design with 3 groups: MBCR, SET, and a mini
mal treatment control group (6-hour stress managemen
 seminar [SMS]). Participants were randomized in co
orts between October 2007 and December 2010. The s
udy was approved by the Institutional Review Board of t
Faculty of Medicine at the University of Calgary. The s
udy was registered on clinicaltrials.gov (number NCT00390169).

Participants

Participants were breast cancer survivors who had com	pleted all medical treatments at least 3 months previou	ly, with the exception of hormonal or trastuzumab th
rapy. Women were included if they: 1) were diagnosed w
AJCC stage I to III breast cancer; 2) were aged >18 yea	s; and 3) scored $4 on the National Comprehensive Can	er Network Distress Thermometer,29 thereby indicating cl
inically significant distress.30 We included only those w
omen who exhibited significant distress because they w	ould likely benefit most from these interventions.

Exclusion criteria included: 1) a diagnosis of a con	current disorder of either psychosis, substance abuse, b
ipolar disorder, or active suicidality (according to the 4
h edition of the Diagnostic and Statistical Manual of Me
Disorders [DSM-IV]; 2) current use of psychotropic medications (antipsychotics or anxiolytics; the use of antidepressants was not an exclusionary factor due to their high prevalence); 3) a diagnosis of a concurrent autoimmune disorder; and 4) past participation in an MBCR or SET group.

A total of 271 women participated in the larger study from both Calgary, Alberta and Vancouver, British Columbia. Due to the availability of resources, blood samples were only collected in Calgary. Of the 128 women in Calgary, 5 declined to donate their blood. Thirty-one women provided their blood only at the preintervention time period; therefore, the current study included 92 women who donated a blood sample before and after the intervention.

Procedures
Participants were self-referred through advertising or direct mailing of personalized study invitation letters through cancer registries. If patients were eligible and provided informed consent, before and after the intervention, they completed questionnaires and donated a blood sample at the cancer center laboratory. Women in the control group provided their blood samples before and 10 weeks after the SMS. Participants did not receive any incentives for their participation.

Randomization and Blinding
Randomization was conducted once a group of participants was assembled for a cohort. The biostatistician randomized participants into one of the MBCR, SET, or control programs with a 2:2:1 ratio using the Research Randomizer (randomizer.org/). The intervention commenced within 2 weeks of randomization. Both participants and Research Assistants were blind to the condition at the time of baseline assessment.

Interventions
Mindfulness-based cancer recovery
MBCR was modeled on the mindfulness-based stress reduction program originally developed at the Massachusetts Medical Center.31 The program cultivates mindfulness, awareness of the present moment in an open and nonjudgemental manner.31 Two of us (L.E.C. and M.S.) developed and manualized MBCR32 specifically to meet the needs of oncology populations, and its efficiency has been validated in previous studies.33-39 Facilitators were clinical psychologists and a nurse who were fully trained in mindfulness-based stress reduction and had led groups in previous MBCR trials. Participants attended 8 weekly group sessions of 90 minutes in duration, and a six-hour retreat between weeks 6 and 7. Participants were provided with a course booklet and practice compact discs for guided meditation and mindful body movement at home.

Supportive-expressive group therapy
SET is also a manualized, well-validated psychosocial intervention for oncology populations.40 Participants met in a group for 90 minutes weekly for 12 weeks with clinical psychologist(s) and/or clinical social worker(s) who were highly experienced in SET. The SET program encourages openness and emotional expression, with an aim toward developing a mutual support system among members as well as improving interactions with family and treating physicians. Through group discussion, SET also aims to facilitate coping skills and to detoxify negative emotions surrounding mortality.

MBCR and SET were different in content; however, both programs were similar in nonspecific components, including structure, group size, group environment, and total contact time (18 hours).

Control Condition
Stress management seminar
Women in the control condition participated in a 1-day (6-hour) didactic SMS led by an experienced clinical social worker. The seminar was the same control condition used by the University of Miami Center for Psycho-Oncology Research.41 The SMS was used to minimize the likelihood of demoralization for those randomized to the control condition and hence to maximize accrual and retention.

Measures
Demographics
Age, marital status, employment status, education history, medical and psychiatric history, current medications, and previous experience with yoga or meditation were obtained.

Disease parameters
Information regarding the stage of disease at the time of study enrollment, first diagnosis date, and types and frequency of medical treatment were obtained through the Alberta Cancer Registry.

Health behavior
Daily physical activity level, alcohol and nicotine intake, and quality of diet and sleep were assessed.

Mood
The Profile of Mood States (POMS)42 assesses 6 dimensions of mood.43 A total score was used to indicate the overall level of mood disturbances.
Stress
The short form of the Symptoms of Stress Inventory (SOSI),\textsuperscript{44} the Calgary SOSI (C-SOSI),\textsuperscript{45} consists of 56 items and 8 subscales. It assesses physical and psychological symptoms and behavioral responses to stressful situations. A total score was used to indicate the level of subjective stress.

Preparation of blood samples
Two 6-mL ethylenediamine tetraacetic acid tubes of blood collected from participants before and after the intervention were refrigerated and processed within 24 hours of collection. The ethylenediamine tetraacetic acid tubes were mixed well, allowed to sit for 30 minutes, and then centrifuged at 1500g for 10 minutes at 4°C. A total of 0.25 mL of buffy coat was aliquoted into each of 4 cryovials and frozen at −80°C. Total genomic DNA was extracted from 250 μL of buffy coat using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, Md), as per the manufacturer’s instructions.

Telomere length
We used a high-throughput analysis to measure relative TL using quantitative real-time polymerase chain reaction (qPCR). Sample reactions were set up in triplicate using 10 ng of template DNA and qPCR was run using Power SYBR Green PCR Master Mix (Life Technologies, Grand Island, NY) for both TL and the single-copy gene, ribosomal acidic protein 36B4. Fluorescent signal detection was monitored and quantified as per the manufacturer’s directions on an ABI7900 Real Time PCR System (Applied Biosystems, Foster City, Calif). A reference sample was run on each reaction plate to calculate interplate variation. The ratio of the telomere PCR signal to the single-copy gene (ribosomal acidic protein 36B4) signal (the T/S ratio) is proportional to the average TL of all the cells in a sample and was based on the calculation of the ΔCt (Ct\textsubscript{telomere}/Ct\textsubscript{single gene}), normalized to the average T/S ratio of a pooled reference standard. The reference sample is the average T/S ratio of all samples run, which by definition relative to itself is 1.00. T/S values, therefore, represent relative TLs, expressed relative to the T/S ratio value of the reference standard DNA sample. Samples with a T/S >1.0 have an average TL that is greater than the standard DNA; samples with a T/S <1.0 have an average TL that is shorter than the standard DNA.

Data Analyses
Potential baseline differences with regard to demographic and medical variables between conditions were assessed. T/S ratio values were determined as outliers and excluded if they were either greater or lower than 4 standard deviations from the mean. Two T/S ratio values were excluded, from each of the pre and post-assessment time points, resulting in a total of 88 survivors with complete data. The T/S ratios were positively skewed. To correct this, log\textsubscript{10} transformations were applied to the pretreatment and posttreatment T/S ratios. To identify potential confounders, either correlations (for continuous variables) or analyses of variance (for categorical variables) were conducted among TL, age, time since diagnosis, alcohol and nicotine intake, cancer severity, quality of sleep and diet, marital status (single, married/cohabitating, or separated/divorced/widowed), and employment status (full-time work, part-time work, or retired/unemployed).

Objective 1
Our primary analysis compared the log\textsubscript{10} T/S ratios of the MBCR and SET psychosocial interventions (the treatment group) with the control group in an analysis of covariance (ANCOVA). We used an orthogonal contrast to make this comparison and used preintervention log\textsubscript{10} T/S ratios as a covariate. Within the same ANCOVA, we first used another orthogonal contrast to test the comparability of postintervention T/S ratios in the 2 intervention arms (MBCR vs SET).

Objective 2
Residual change scores were calculated by obtaining residual scores from linear least-squares models using pretreatment values to predict posttreatment values for the posttreatment log\textsubscript{10} T/S ratio, POMS total score, and the C-SOSI scores, using pretreatment scores on the same variable as predictors. Using residualized change scores is hence a much more conservative analysis than using raw pre to post values. We then calculated Pearson product-moment correlations among residual change scores both within each treatment group and for all subjects (ignoring treatment group). In all analyses, 2-sided alpha values of 5% were used. All statistical analyses were conducted using IBM SPSS software (version 20; SPSS Inc, IBM Corporation, Armonk, NY).

RESULTS
Participants
Figure 1 shows the flow of participants. Table 1 summarizes demographic and medical characteristics for all groups. The 3 groups were well balanced with regard to their baseline characteristics, with no group differences found.
Confounding Variables
There were no associations noted between baseline TL and any of the medical, demographic, and health behavioral factors (all $P > .19$). Hence, only baseline TL was included as a covariate in the subsequent analyses.

Objective 1: Changes in TL Between MBCR Versus SET
Table 2 shows TL at both preintervention and postintervention across groups and the results of significance testing. Figure 2 represents distributions of preintervention and postintervention T/S ratios across groups. The results of ANCOVA demonstrated no statistical evidence of differences in postintervention TL between the MBCR and SET interventions after adjusting the impact of the preintervention log10 T/S ratios. The mean difference was $-0.12$ (95% confidence interval [95% CI], $-0.74$ to 0.50). Because the 2 interventions shared similar nonspecific components and no significant differences emerged in their baseline-adjusted postintervention T/S ratios, the 2 intervention groups were subsequently combined to allow greater power for detecting any effects on TL related to participation in a psychosocial intervention compared with the control condition.

Changes in TL Between Intervention Versus Control
After adjustment for the baseline log10 T/S ratio, there was a statistical trend toward a difference in posttreatment log10 T/S ratios between treatment and control subjects (statistics shown in Table 2). The adjusted mean difference was 0.67 (95% CI, $-0.01$ to 1.35). The effect size of $\eta^2$ was 0.043 (small to medium). T/S ratios in the control group demonstrated a trend toward a decrease
relative to those in the intervention group, which remained relatively stable.

Based on these results, we calculated a sample size to detect a significant group difference with 80% power. Although the exact size of the change in T/S ratios needed for clinical significance is unknown, to detect a 0.5 difference in T/S ratios between the intervention and the SMS, a new trial would require 106 survivors in each group (total of 212 survivors) with a 1:1 randomization ratio.

Objective 2: Associations Between TL and Psychosocial Measures

There was no statistical evidence of correlations between residual changes in TL and POMS or C-SOSI in the intervention ($r = -0.03$ [P = .804] and $r = 0.14$ [P = .244]) or control ($r = -0.27$ [P = .277] and $r = -0.11$ [P = .669]) groups, or across the 2 combined conditions ($r = -0.11$ [P = .307] and $r = 0.07$ [P = .546]).

DISCUSSION

To our knowledge, the current study is the first report to demonstrate a potential effect of these psychosocial interventions on TL among distressed breast cancer survivors. There was little discernment between MBCR and SET. Both maintained TL over the 3-month intervention period, whereas women in the control condition demonstrated a trend toward decreases in relative TL. Similarly, in our previous report of the larger parent trial, both the MBCR and SET groups maintained the steepness of salivary cortisol slopes compared with those in the control group, whose slopes became flatter, largely due to elevations in evening cortisol levels. Together, these changes suggest an effect of the interventions on potentially important biomarkers of psychosocial stress. Given the increasingly well-documented association between TL and cancer initiation and survival, this finding adds to the literature supporting the potential for stress-reducing interventions to impact important disease-regulating processes and ultimately disease outcome.

Although the current study is strengthened by randomization and the inclusion of only distressed survivors, it does have several limitations. Chief among these is missing data, which precluded the feasibility of conducting intent-to-treat analyses. The control condition was also therefore small (18 individuals), because twice as many women were randomized to the active intervention groups as to the control condition. Hence, the study would

| TABLE 1. Baseline Demographic and Medical Characteristics of Participants Across 3 Conditions |
|-----------------------------------------------|-------------------|-------------------|-------------------|
| Characteristics                              | MBCR n = 34       | SET n = 36        | Control n = 18    |
| Mean age (SD), y                              | 54.43 (8.00)      | 54.05 (9.50)      | 56.01 (10.20)     |
| Mean no. of y of education (SD)*              | 14.81 (2.44)      | 15.27 (3.09)      | 13.78 (1.90)      |
| Mean time since diagnosis (SD), mo            | 25.56 (24.33)     | 27.74 (35.94)     | 21.16 (14.49)     |
| Marital status, no. (%)*                      |                   |                   |                   |
| Single                                        | 2 (5.9)           | 2 (5.6)           | 1 (5.6)           |
| Cohabiting/married                            | 28 (82.4)         | 24 (66.7)         | 14 (77.8)         |
| Divorced/separated/widowed                    | 4 (11.8)          | 9 (25.0)          | 3 (16.7)          |
| Employment, no. (%)#                          |                   |                   |                   |
| Unemployed/retired/disabled                   | 14 (41.2)         | 14 (38.9)         | 8 (44.4)          |
| Part time                                     | 8 (23.5)          | 8 (22.2)          | 2 (11.1)          |
| Full time                                     | 12 (35.3)         | 13 (36.1)         | 8 (44.4)          |
| Cancer stage, no. (%)#                        |                   |                   |                   |
| 0                                             | 1 (2.9)           | 1 (2.8)           | 2 (11.1)          |
| I                                             | 14 (41.2)         | 18 (50.0)         | 7 (38.9)          |
| II                                            | 13 (38.2)         | 11 (30.6)         | 7 (38.9)          |
| III                                           | 4 (11.8)          | 3 (8.3)           | 2 (11.1)          |
| Medical treatment received, no. (%)           |                   |                   |                   |
| Surgery only                                  | 0 (0)             | 3 (8.3)           | 0 (0)             |
| Surgery and radiation                         | 2 (5.9)           | 6 (16.7)          | 1 (6.6)           |
| Surgery and multimodal                        | 32 (94.1)         | 27 (75.0)         | 17 (94.4)         |

Abbreviations: MBCR, mindfulness-based cancer recovery; SET, supportive-expressive group therapy; SD, standard deviation.

*Data were missing for 1 participant.

#Data were missing for 5 participants.
require approximately twice as many participants in each group to detect a change in the T/S ratio of 0.5.

There have also been reports that TL varies with breast cancer subtype, such that women with more aggressive types, including luminal B, human epidermal growth factor receptor 2 (HER2)-positive, and triple-negative tumors, had shorter TL. Unfortunately we did not have subtyping data available. TL also may be affected by chemotherapy among patients with breast cancer, but effects varied across participants in previous research. Some demonstrated increased leukocyte TL after chemotherapy, and some showed decreases, such that no overall group changes were noted. This is consistent with the current study finding of no differences in TL across treatment groups.

We also are not aware of the long-term effects of these interventions on TL. Future investigators should power studies of intervention effects on TL and telomerase as primary outcomes, and follow participants over time to better understand the clinical implications of group differences. The interpretation of any changes in TL in patients with breast cancer is difficult. One study that analyzed TL in breast tumor tissue found no relations between TL and any clinical or pathological features or

| TABLE 2. Preintervention and Postintervention T/S Ratio Values Across Groups and Results of Significance Testing |
|---|---|---|---|---|
| | MBCR and SET F (1, 84), P | MBCR Versus SET F (1, 84), P | MBCR | SET |
| T/S Ratio Measures (Log10 Values) | | | n = 34 | n = 36 |
| Preintervention, mean (95% CI) | -0.27 to 0.54 | 0.14 (95% CI) | -0.27 to 0.54 | 0.14 (95% CI) |
| Postintervention, mean (95% CI) | -0.51 to 0.43 | -0.04 (95% CI) | -0.51 to 0.43 | -0.04 (95% CI) |
| Postintervention T/S ratio mean (SD) | 0.07 (0.35 to 0.48) | 0.04 (0.35 to 0.48) | 0.07 (0.35 to 0.48) | 0.04 (0.35 to 0.48) |

Abbreviations: 95% CI, 95% confidence interval; MBCR, mindfulness-based cancer recovery; SET, supportive-expressive group therapy; SD, standard deviation; T/S ratio, telomere/single-copy gene ratio.

**Figure 2.** Preintervention and postintervention untransformed telomere lengths are shown. For each group, the top and bottom of the box represents the 75th and 25th percentiles of the data, respectively. The horizontal line through the box is the median. The top and bottom of the error bars are the maximum and minimum values, respectively, that are not outliers (the top and bottom 25% of scores). Circles outside of the lines represent individual outliers. T/S ratio indicates the telomere/single-copy gene ratio.
disease or survival outcomes, whereas other studies have shown that TL was related to breast cancer risk and survival. Although interpretation remains difficult, the results of the current study nonetheless provide provocative new data that suggest it is possible to influence TL in cancer survivors through the use of psychosocial interventions involving group support, emotional expression, stress reduction, and mindfulness meditation.

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**CONFLICT OF INTEREST DISCLOSURES**

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