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Reduction in Salivary α -amylase Levels following a Mind-Body Intervention in Cancer Survivors - an Exploratory Study

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Abstract

Objective—The main aim of this exploratory study was to assess whether salivary α -amylase (sAA) and salivary cortisol levels would be positively modulated by sleep-focused mind-body interventions in female and male cancer survivors.

Methods—We conducted a randomized controlled trial in which 57 cancer survivors with self-reported sleep disturbance received either a Sleep Hygiene Education (SHE; n=18) control, or one of two experimental mind-body interventions, namely, Mind-Body Bridging (MBB; n=19) or Mindfulness Meditation (MM; n=20). Interventions were three sessions each conducted once per week for three consecutive weeks. Saliva cortisol and sAA were measured at baseline and one week after the last session. Participants also completed a sleep scale at the same time points when saliva was collected for biomarker measurement.

Results—Our study revealed that at post-study assessment, mean sAA levels upon awakening (“Waking” sample) declined in MBB compared with that of SHE. Mean Waking cortisol levels did not differ among treatment groups but declined slightly in SHE. Self-reported sleep improved across the three interventions at Post-assessment, with largest improvements in the MBB intervention.

Conclusion—In this exploratory study, sleep focused mind-body intervention (MBB) attenuated Waking sAA levels, suggesting positive influences of a mind-body intervention on sympathetic activity in cancer survivors with sleep disturbance.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

Contributors

Author Lipschitz conducted and helped design the study, undertook the statistical analyses and wrote the manuscript. Author Kuhn helped conduct the study. Author Kinney helped with the study design. Author Donaldson oversaw the statistical analyses and approved the final manuscript. Author Nakamura initiated and designed the study and approved the final manuscript.

Keywords

biomarker; salivary α -amylase; salivary cortisol; cancer survivor; mind-body intervention; sleep disturbance; stress; sympathetic nervous system

1. Introduction

Sleep disturbance is a major adverse health condition in cancer patients, affecting a high percentage (30 - 59%) of this population (Savard and Morin, 2001; Palesh et al., 2010; Savard et al., 2011; Sharma et al., 2011). Similarly, a large number of post-treatment cancer survivors (20% or greater) also experience sleep disturbance, which in many cases may continue for several years after cancer diagnosis and treatment (Shapiro et al., 2003; Miller et al., 2008; Savard et al., 2011; Sharma et al., 2011). Psychological factors such as stress, anxiety and depression may strongly influence sleep problems in cancer patients and survivors (Garland et al., 2011) and reduce quality of life (Dodd et al., 2001; Dodd et al., 2010; Lengacher et al., 2011). In addition, in this population, physiological and hormonal stress-related systems may become dysregulated with further negative impact on sleep, quality of life, and well-being. For example, breast cancer patients with sleep disturbance display elevated levels of cortisol and norepinephrine, accompanied by increased SNS activity and increased 24-hr metabolic rate, indicating physiological hyperarousal (Carlson et al., 2007a). Even though sleep disturbance is a persisting problem, it may not always be dealt with effectively in post-treatment cancer survivor care (Bower, 2008; Ancoli-Israel, 2009).

As an alternative to conventional treatments for sleep disturbance in cancer patients and survivors (besides in many other clinical populations), non-pharmacological or behavioral interventions such as mind-body therapies have increasingly shown promise (Carlson and Bultz, 2008; Kwekkeboom et al., 2010). Mind-body therapy or mind-body medicine refers to cognitive or behavioral techniques that improve physical functioning and promote health (NCCAM). Mindfulness training uses the power of mental training to improve health conditions (Begley, 2007). A number of mindfulness training programs have been developed, including Mindfulness-Based Stress Reduction (MBSR) (Kabat-Zinn, 1982; Kabat-Zinn et al., 1985), Mindfulness-Based Cognitive Therapy (Teasdale et al., 2000; Teasdale et al., 2002), and more recently Mind-Body Bridging (MBB) (Block and Block, 2007). Given that sleep problems can exacerbate many other health conditions, sleep-focused mindfulness programs have been developed, including MBSR I-CAN-SLEEP (Garland et al., 2011), and MBB for sleep disturbance (Nakamura et al., 2011). Understanding whether and how these mindfulness programs may help treat and manage sleep disturbance in many populations with medical and psychiatric illnesses, including cancer, will provide information about potentially important alternatives to conventional treatments (Winbush et al., 2007; Shapiro and Carlson, 2009; Fjorback et al., 2011; Nakamura et al., 2011).

Assessment of mindfulness training programs has been generally based on validated self-reported outcome measures, which have shed light on how mindfulness training may influence and improve psychological states and symptoms, including quality of life, well-being, resilience, and health symptoms specific to conditions such as insomnia, depression, hypertension, gastro-intestinal disorders, and cancer. More frequently, studies are investigating the effects of mindfulness programs on physiological systems, including cardiac functioning (measurements include heart rate, blood pressure, heart rate variability), and the endocrine (hormones) and immune (cytokines) systems.

In the past, biological substances were measured in plasma and/or urine. More recently, saliva has been favored as a non-invasive approach to evaluate a variety of biological substances, including steroids such as cortisol and testosterone, salivary immunoglobulin A (SIgA) and salivary α -amylase (Papacosta and Nassis, 2011). Salivary measures are increasingly used in wide ranging bio-behavioral and clinical research to understand the impact of stress on psychological health and disease progression, including cancer. Salivary cortisol in particular is a frequently measured and well-characterized hormone activated via the hypothalamic-pituitary-adrenal cascade, and a large body of research has evaluated collection and measurement methodologies, and outcomes using cortisol as an indicator of physiological stress (Kirschbaum and Hellhammer, 1994; Adam and Kumari, 2009). While other stress hormones, epinephrine and norepinephrine, are not found in saliva in reliable quantities, an enzyme secreted in saliva, salivary α -amylase (sAA), is stimulated by the autonomic nervous system (ANS), which also controls the salivary glands. Thus, sAA may provide an appropriate measure of sympathetic activity (see Nater and Rohleder, 2009, for a review). Since cortisol and sAA are released from different hormonal systems, and have divergent patterns of diurnal secretion (Nater et al., 2007; Wolf et al., 2008), they highlight different aspects of stress regulation specifically appropriate in the context of clinical populations.

In addition to the impact of cortisol and sAA on stress, these biomarkers affect and are affected by sleep, since deprived or disrupted sleep may also act as a stressor. Studies have shown that elevated plasma cortisol and norepinephrine are associated with sleep deprivation (reviewed in Meerlo et al., 2008). However other studies have found that both cortisol and norepinephrine are lower in people with insomnia, perhaps associated with excess daytime sleepiness (Meerlo et al., 2008). In either case, it appears that sleep disturbance negatively impacts diurnal patterns of cortisol and norepinephrine, and likely by association, sAA.

A number of studies have documented the effects of mindfulness training programs in cancer patients and survivors using biomarker measures, although few have documented changes in sleep and concomitant effects on stress-related biomarkers. One of the more prolific mindfulness programs, MBSR, has been associated with reductions in salivary cortisol at post-intervention in cancer patients (Carlson et al., 2004; Carlson et al., 2007b). However, MBSR has not always lead to any change in salivary cortisol levels at post-intervention (Matousek et al., 2010), although methodological shortcomings could account for this lack of effect (Matousek et al., 2010). Similarly, a review of the literature indicates that the effects of mindfulness training programs on sAA have not been extensively investigated, and that there is little research specific to cancer patients and/or cancer survivors. Drawing on the more general literature investigating the experimental or interventional effects on sAA, in one study a “warm touch” intervention with young couples conducted over four weeks was associated with reductions in sAA at post-intervention compared with that of the control group (Holt-Lunstad et al., 2008). In another study, stress management conducted over the course of eight months decreased sAA indices one year later, compared with that of a waitlist control (Limm et al., 2011). Thus, more studies are required to determine the effects of mindfulness training programs targeting sleep disturbance and stress on cortisol and sAA in cancer survivors.

In the present exploratory study, we evaluated the effects of two sleep-focused mind-body interventions, Mind-Body Bridging (MBB) and Mindfulness Meditation (MM) compared with that of Sleep Hygiene Education (SHE), on biological indicators of stress, namely salivary α -amylase (sAA) and salivary cortisol. We included a self-reported sleep outcome measure to determine whether potential alterations in biomarkers may be correlated with changes in sleep patterns. We have already shown in cancer survivors with sleep disturbance

that MBB and MM improved sleep in comparison with that of SHE, while MBB also increased self-reported mindfulness, self-compassion and well-being and decreased self-reported depressive symptoms (manuscript under review). Given these improvements in sleep and related psychological variables, we hypothesized that the mind-body interventions would attenuate levels of sAA and cortisol to a greater extent than would the control intervention. Thus, at the post-assessment we predicted that in the mind-body interventions, sAA and cortisol would show reduced Waking levels, lower diurnal profiles and lower daily output, associated with reductions in self-reported sleep disturbance. Our rationale for expecting reductions in these biomarkers is that post-treatment cancer survivors may not be as chronically stressed as cancer patients who are still undergoing cancer treatment.

2. Methods

2.1. Participants and recruitment

Participants included females and males, 18-75 years old, recruited primarily from Huntsman Cancer Institute (University of Utah), oncologists in private practice, and a non-profit organization, Cancer Wellness House serving as a cancer support center. The study was conducted from July 2009 through April 2010 and the interventions were conducted at the Cancer Wellness House. Primary inclusion criterion was presence of self-reported sleep problems as indicated by a score of 35 or greater on the Medical Outcomes Study Sleep Scale (MOS-SS) using the Sleep problems Index II subscale (SPI-II; Hays et al., 2005). Any type of sleep disorder was allowed. Other inclusion criteria were: any cancer diagnosis, post-treatment (surgery, chemotherapy, or radiation), in remission > 3 months by oncologist assessment, English speaking. Adjuvant hormone therapy (e.g., Tamoxifen, Lupron, or aromatase inhibitors) was allowed, as were any sleep medication or other medication not explicitly listed in the exclusion criteria, below. Participants were excluded from the study if they demonstrated any severe psychopathology or psychiatric illness (e.g., schizophrenia), cognitive impairment, neurologic disorders, or dementia, were on antipsychotic medication, immuno-suppressants, or corticosteroids, had metastatic disease, or had previous exposure to MBSR, MBB or any other mindfulness-based program employing similar techniques. Exclusions were determined by contacting the potential participant's oncologist or other primary provider, who were specifically asked to complete a brief questionnaire indicating if the person presented with any of the psychiatric or medical exclusions.

2.2. Study Design

The original study was a prospective, randomized trial investigating the effects of two brief sleep-focused mind-body interventions compared with a sleep hygiene education control on sleep performance and other psychological outcomes in cancer survivors. In the present exploratory study we investigated the effects of these sleep-focused mind-body interventions on biological measures of stress, in the identical population of cancer survivors. Interventions were three sessions of two hours' duration each conducted once a week for three consecutive weeks. We randomly assigned participants to either the Sleep Hygiene Education program (SHE; control; n=18), or to one of the two experimental programs, Mind-Body Bridging (MBB; n=19) or Mindfulness Meditation (MM; n=20), using computer-generated numbers.

2.3. Interventions

a. Sleep Hygiene Education Program (SHE; treatment as usual)—Participants were informed about ways to change their daily activities and evening habits to improve their sleep. They were also given suggestions for reducing their concerns about problems with their sleep quality. The program was based on the Huntsman Online Patient Education (HOPE) Guide, "Insomnia: Patient Symptom Information" (<http://www.hopeguide.org/>

hope/). Topics discussed included: “What would cause my insomnia?” (medications, stress, anxiety, etc), “When should I speak with my doctor about my sleep disturbance?” (Worries/concerns about sleeping and having questions about insomnia), “What behavioral changes would help me to get a better night’s sleep?” (Diet, exercise, supportive care, getting ready for bed), “What activities to avoid to get a better night’s sleep?” (Tips on behaviors that can negatively influence sleep quality), and “Medications used for sleep problems?” (Side-effects and of sleep medications; the use of other medications affecting sleep). As part of the program, participants could ask questions and the group discussed their sleep issues. Participants were encouraged to implement the SHE recommendations each day. SHE classes were presented by a licensed clinical social worker who is an experienced social worker in the field of cancer supportive care.

b. Mindfulness Meditation Program (MM)—MM was based on Mindfulness-Based Stress Reduction (MBSR) (Kabat-Zinn, 1991), which is an 8-week program teaching awareness and mindfulness skills, including basic meditation practice and yoga. Sleep-focused MBSR programs have tested a 6-week long format (Shapiro et al., 2003; Lengacher et al., 2009). In the present study, the three-week MM course was an adaptation of some of the activities in MBSR, including breath meditation, body-scan meditation, and walking meditation. The MM program comprised the following: mindfulness meditation at each weekly session, learning mindfulness meditation skills including breath awareness and awareness of thoughts and emotions, walking meditation, and forgiveness meditation. The group also engaged in a body scan in which they experienced each part of their body while lying down and the instructor asked them to pay attention to each part of their body she mentioned. The group also engaged in conversation during which they discussed their cancer experiences and sleep difficulties. However, discussion about their cancer was only related to the mindfulness meditation practices. They also engaged in group discussions of how to implement moment-to-moment awareness in their daily lives, and how to integrate mindfulness throughout the day. Discussion of sleep issues was specifically related to mindfulness processes taught in class. They were encouraged to practice assignments at home, but at their own discretion. They were provided MBSR meditation CDs covering body scan and breath meditation, to practice throughout the day or at night time as their schedules allowed. Although MBSR comprises yoga practice, no yoga was taught, given that there were three weekly sessions compared with MBSR’s 8 weekly sessions. Participants were also asked to write about anything that they felt was causing them to feel stressed, with a focus on including emotions and expressing themselves as fully as possible. Handouts for the course included the CDs for mindfulness meditation, a copy of the book, *Full Catastrophe Living*, (Kabat-Zinn, 1991) a printout on “The Practice of Mindfulness” taken from the book, and stress reduction tips. MM was taught by a licensed clinical social worker, who is also an oncology social worker and an experienced MBSR instructor with years of meditation experience.

c. Mind-Body Bridging Program (MBB)—A summary of MBB is more extensively reported in previous articles (Tollefson et al., 2009; Nakamura et al., 2011). Briefly, MBB is a mind-body intervention teaching awareness skills and “mapping” exercises to help individuals recognize and become aware of a dysfunctional mind-body state characterized by heightened self-centeredness. Mind-body “mapping” is designed to reveal counter-productive thought patterns known as “requirements,” which are expectations about how people and the world should be at particular moments (an example of a requirement is “I should be less stressed about recovery from cancer”). When requirements are not fulfilled, people may develop excessive rumination building into storylines, which could lead to an increase in both psychological and physiological stress levels and dysfunctional mind-body states. By using awareness practices and defusing requirements through mapping exercises,

MBB can help patients expand awareness in order to deal more effectively with life's daily challenges. As part of the focus on sleep, MBB training included the following: identifying the underlying source of sleep problems from an MBB perspective; learning to use MBB tools, such as awareness exercises to reduce daytime stress; addressing sleep issues pertinent to cancer survivors; and practicing MBB on a daily basis. Participants were provided a MBB published book, *Come to your senses* (Block and Block, 2007), which they were encouraged to read and practice MBB techniques and exercises. MBB was taught by a licensed clinical social worker with certification and extensive expertise in the MBB program.

Adherence to programs—Participants in all groups were advised to use the techniques and the information they learned in their daily lives. In the MM group, they were encouraged to additionally listen to the CDs and look over the two books used in the course. In the MBB group, they were asked to complete mind-body mapping exercises from the textbook used. In the SHE group, they were asked to review periodically what they learned from the SHE program.

2.4. Study Procedures

Potential participants completed a screening questionnaire asking about sleep patterns, demographics, cancer history (cancer site and treatment), other medical history and what medications and supplements they were currently taking. A person was provisionally eligible based on their scoring 35 or greater on the MOS sleep scale (MOS-SS SPI-II), and their fulfilling other inclusion criteria (see above). To ensure eligibility, and with the participant's signed consent, we contacted their oncologist or other health care provider who was asked to complete a brief questionnaire in which they confirmed that their patient's mental health and remission status satisfied inclusion/exclusion criteria. Successful participants enrolled in the study by signing an informed consent form. The Institutional Review Board at the University of Utah approved the study protocol.

2.5. Outcome measures

Participants were assessed for study outcome measures using several self-report questionnaires, including MOS-SS, as reported elsewhere (manuscript under review), and the salivary measures, salivary α -amylase (sAA) and cortisol. In this article, we provide self-reported data only for the primary outcome measure, MOS-SS, besides for sAA and cortisol. Saliva samples and the MOS-SS questionnaires were collected at baseline (within one week prior to the first session; PRE) and at post-study (a minimum of one week following the final session; POST). Saliva samples collected at these time points generally corresponded to collection of self-reported questionnaires by participants.

Biomarkers—Cortisol and sAA were sampled at standardized times over the course of a 24 hr period. Participants were asked to collect saliva samples the first day at: a. Post-awake (30 min. after awakening), b. Noon, c. Afternoon (~5 pm), d. Evening (before going to bed, ~10 pm), and e. Waking (the following morning upon awakening, before getting out of bed). This procedure made it easier for participants to collect the first sample after they got out of bed, but it precluded calculation of the awakening responses for cortisol and sAA. Samples were collected using the Salivette collecting device (Sarstedt, Inc., Newton, North Carolina), which is a commonly used saliva sampling technique, with minimum invasiveness (Granger et al., 2007). Participants received standard instructions for collecting saliva samples based on descriptions provided in Holt-Lunstad et al. (Holt-Lunstad et al., 2008).

Compliance with saliva collection: Since we did not have the resources to determine compliance with saliva collection using an electronic device, we asked participants to record

the time of saliva collection on their saliva collecting tubes or on the instruction sheet. We could not confirm that they strictly adhered to the times we requested them to collect saliva.

Cortisol and sAA assay procedures employed in the present study are described in detail in (Strahler et al., 2010). Briefly, saliva samples were stored in a freezer (−20°C) until shipped on dry ice for assay. After thawing, salivettes were centrifuged at 3000 rpm for 5 minutes, which resulted in a clear supernatant of low viscosity. Salivary free cortisol was measured using a commercial immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Germany). The assay has a lower detection limit of 0.1 nmol/l with intra- and interassay coefficients of variations <8%. Salivary α -amylase was measured by a quantitative enzyme kinetic method. Intra- and inter-assay precision expressed as percent coefficient of variation was below 10%.

Sleep Outcome Measure—Medical Outcomes Study Sleep Scale (MOS-SS; Hays et al., 2005): The MOS-SS is a validated scale comprising 12 items, including questions about sleep disturbance, sleep adequacy, daytime somnolence, snoring, waking up short of breath or with a headache and sleep quantity. MOS-SS has been used as an instrument to investigate disrupted sleep in cancer populations (Bower et al., 2000; Thomas et al., 2010). It is validated for a one-week recall, which is what we used in the present study and a previous study (Nakamura et al., 2011).

We evaluated changes in sleep using the same two time points analyzed for salivary cortisol and sAA, namely baseline (PRE) and post-assessment (POST). In the present study, we assessed changes in sleep disturbance using the Sleep Problems Index II (SPI-II) subscale of MOS-SS, a composite sleep measure incorporating sleep disturbance, sleep adequacy and somnolence. Higher values indicate greater sleep problems, and a cut-off of 35 indicates a strong likelihood that an individual has clinical sleep disturbance (see Nakamura et al., 2011).

2.6. Statistical analysis

All data analyses were conducted with SPSS software, Version 18.0. The analyses evaluated the effects of the mind-body programs (MM, MBB) compared with that of SHE, on sAA and salivary cortisol measures as well as on the self-reported MOS-SS Sleep Problems Index-II (SPI-II), at POST.

Salivary alpha-amylase and cortisol—For evaluating sAA and cortisol levels, we conducted the statistical analyses using three measures: a. Waking sample (collected upon awakening before participants got out of bed), b. Area under the curve (AUC), comprising four saliva samples collected on the same day (Post-awake, Noon, Afternoon, and Evening), c. Diurnal profile, comprising the same four collections in a single day (see above). Since cortisol and sAA levels were positively skewed, we normalized the data using a log transformation.

a. Waking sample: For sAA and cortisol levels measured in saliva samples collected upon awakening, we conducted a mixed effects linear model analysis of covariance (ANCOVA) using baseline (PRE) as a covariate, for comparisons among interventions at POST. The fixed factor was Intervention. This approach of using the baseline as a covariate makes it possible to directly compare POST levels across the 3 interventions, and is similar to the practice of normalizing the data, but incorporates a more sophisticated mixed-models analysis.

b. Area under curve (AUC): The AUC is considered a measure of total biomarker daily output (Pruessner et al., 2003). The AUC for cortisol and sAA, comprising the four samples collected over the course of the day, was derived from the trapezoid formula and was calculated according to the approach recommended by Pruessner et al. (2003). Since collection times were not evenly spaced, we used AUC with respect to increase (AUCi) (Pruessner et al., 2003). We employed a mixed effects model (ANCOVA) with baseline (PRE) as covariate to evaluate differences among interventions at POST. The fixed factor was Treatment.

c. Analysis of diurnal profile: We employed a mixed effects model ANCOVA for sAA and cortisol levels at POST using the baseline (PRE) level as a covariate. Intervention, Collection Time and Intervention by Collection Time interaction were the fixed factors. Note that since we collected the Waking sample on the following day, we did not include this measure in the analysis of diurnal profiles of sAA and cortisol.

Sleep Measure—For the Medical Outcomes Study - Sleep Scale (MOS-SS), we conducted a mixed effects linear model ANCOVA, using the baseline score as a covariate, on MOS-SS Sleep Problems Index-II (SPI-II) subscale at POST with Treatment as the fixed factor.

An “intent-to-treat” analysis was done for all of the mixed models analyses. Thus, data were included from all participants, regardless of whether they completed the study, because the mixed effects model is able to handle missing observations without discarding or imputing data.

3. Results

Demographics and clinical characteristics

Table 1 presents demographic and clinical characteristics of the cancer survivors enrolled in our study. Most of the participants were non-Hispanic white females with a prior diagnosis of breast cancer. Only two females and one male were Hispanic. As seen in the Table 1, groups were fairly well balanced with regard to age, gender and clinical characteristics.

Baseline diurnal sAA and cortisol patterns

Figure 1 depicts mean diurnal levels of sAA and cortisol at baseline (PRE) comprising five collection time points over a 24 hr. period. The first day comprised four collection samples, Post-awake (30 min. after awakening), Noon, Afternoon, Evening, and the second day comprised the Waking sample (collected upon awakening). Since there were no significant differences in sAA and cortisol levels between females and males, their data were pooled (data not shown). As can be seen in Figure 1, sAA levels increased from Post-awake to Noon, which was generally maintained for the remainder of the day. The mean Waking sAA level on the second day was greater than the mean Post-awake level on the first day, but an awakening response could not be computed due to the ~24 hr separation between them. In Figure 1, mean cortisol levels were greatest at Post-awake with a gradual decline throughout the day until reaching a nadir in the Evening. The Mean Waking cortisol level on the second day was slightly greater than the first day's mean Post-awake cortisol level, but as in the case of sAA the awakening response could not be computed due to the ~24 hr separation between them.

In the following section, we present the effects of the mind-body interventions on 1) sAA and cortisol levels and 2) sleep performance.

Salivary alpha-amylase and cortisol measures

a. Waking sample—Figure 2a and b show raw mean Waking sAA and cortisol levels, respectively, depicting the effects of the interventions on sAA and cortisol at post-study assessment (POST). For Waking sAA, the ANCOVA generated $p=.054$ for the main intervention effect. Specifically, the mean sAA level in MBB was lower than that in SHE ($p=.019$) while MM and SHE did not differ ($p=.371$). For within group comparisons, only the MBB intervention showed a decrease in the mean Waking sAA level from PRE to POST ($p=.010$). Table 2 presents raw mean Waking sAA levels for each intervention at the PRE and POST assessments.

Mean Waking cortisol levels among interventions did not differ based on the ANCOVA ($p=.147$). As shown in Figure 2b, the mean Waking cortisol level for SHE was slightly lower than that for both MBB ($p=.083$) and MM ($p=.092$). For within group comparisons, only the SHE intervention showed a decrease in the mean Waking cortisol level from PRE to POST ($p=.052$). Table 2 presents raw mean Waking cortisol levels for each intervention at the PRE and POST assessments.

b. AUCi—the ANCOVA did not discriminate among the three interventions for AUCi of sAA ($p=.989$) or cortisol ($p=.555$). Table 2 presents unadjusted AUCi mean sAA and cortisol levels for each intervention at PRE and POST assessments.

c. Diurnal Profiles—Table 3 presents raw mean diurnal sAA and cortisol levels for Post-awake, Noon, Afternoon and Evening for each intervention at PRE and POST. The ANCOVA for diurnal mean sAA levels revealed that there were no differences among interventions ($p=.600$) or in the intervention by collection time interaction ($p=.973$). Similarly, the ANCOVA for mean diurnal cortisol levels showed no effect of intervention ($p=.188$) or intervention by collection time interaction ($p=.406$). However, for the collection time variable, mean cortisol levels declined to a significant extent over the day ($p<.001$).

Sleep Measure

Figure 3 shows raw mean sleep problems index-II scores depicting effects of the interventions on sleep at POST. Based on the ANCOVA, mean sleep scores differed among the interventions ($p=.005$). Specifically, the mean sleep score in MBB was lower than that in SHE ($p=.001$), while MM and SHE did not differ ($p=.150$). For within group comparisons, all interventions showed a decrease in mean sleep scores from PRE to POST (SHE, $p<.01$; MBB, MM, $p<.001$; see Table 2).

Multivariate Analysis

To evaluate potential relationships among the three variables (Waking sAA, Waking cortisol, and the sleep measure) we conducted a multivariate ANCOVA (MANCOVA) using the three dependent variables' baseline values as covariates. We tested the null hypothesis that the adjusted means at POST are equal across the three interventions for the three variables. We also examined correlations among the three variables adjusted for their respective covariates. The multivariate simultaneous test using the three variables rejected the null hypothesis of equal intervention population mean vectors, conditional on baseline vectors ($p=.008$), that is, adjusted means differed among the three interventions. However, correlations at POST among sAA, cortisol and sleep scores were weak ($r < .1$).

4. Discussion

The aim of this study was to evaluate whether two sleep-focused mind-body interventions (MBB and MM) could alter profiles of salivary α -amylase (sAA) and cortisol release in a

group of cancer survivors with sleep disturbance. The study documented that MBB was effective in reducing Waking sAA levels as compared with those of the control intervention (SHE). In contrast, there were no apparent effects of the mind-body interventions on salivary cortisol levels. While self-reported sleep disturbance decreased significantly in all groups, MBB demonstrated a more pronounced reduction in sleep disturbance compared with that of SHE. These results suggest that the Waking sAA may be an appropriate measure to use in examining the effects of mind-body interventions on sleep disturbance.

The main finding of the analysis was a reduction in Waking sAA levels following MBB compared with that of the control group. Salivary α -amylase is characterized as a proxy measure of stress-induced changes in the body, associated with increased sympathetic activity (reviewed in Nater and Rohleder, 2009). This suggests that MBB may have influenced the reduction in sympathetic activity at the time of awakening, as indexed by sAA. While the Waking measure per se is not the one that is most commonly assessed, it could serve as a useful marker to investigate potentially clinically relevant health outcomes (Adam and Kumari, 2009; Kumari et al., 2009). In more general terms, reductions in Waking sAA is consistent with the findings of a few studies examining longer-term effects of stress-reduction interventions on sAA. These studies demonstrated that tactile contact (“warm touch”) between couples (Holt-Lunstad et al., 2008), or a stress management intervention (Limm et al., 2011) in healthy adults, was associated with declines in sAA measures at post-intervention assessments (4 weeks and 1 year, respectively). Thus, our results provide evidence that sAA might be an appropriate biomarker to evaluate intervention effects on sleep disturbance and related physiological measures of stress.

Using a multivariate approach (MANCOVA), we additionally showed that adjusted mean levels for the two biomarkers and the sleep variable evaluated together were not equal across interventions. Although both Waking sAA and the sleep measure went in the same direction for MBB, that is, they both declined at post-assessment, correlations between them were weak. Lack of correlation between the two could be due to differences in recall time of the sleep questionnaire (7 days previously), compared with a more proximate measurement of the Waking sample in the case of the biomarkers. Future studies should include the collection of sleep information as close as possible to the time at which the morning saliva sample is collected. This may help identify whether sAA (and possibly cortisol) could be more strongly associated with self-reported sleep data. Although our findings did not indicate a strong relationship between the two waking biomarkers and the self-report sleep scale, it may be helpful in future to elucidate a possible dynamic interplay among biomarkers and self-report measures.

It is uncertain why salivary cortisol was not altered by either of the mind-body interventions as shown for sAA. Furthermore, it is unclear why SHE showed some (albeit small) decrease in the Waking sample for cortisol compared with a lack of change for that measure in the mindfulness programs. Some studies have demonstrated the effects of mind-body therapies in decreasing salivary cortisol levels in an eight week program (Carlson et al., 2004; Carlson et al., 2007b), whereas our interventions were brief (three sessions spaced once per week over three consecutive weeks). However, other studies have indicated lack of change in cortisol, possibly reflecting methodological issues (reviewed in Matousek et al., 2010), such as small sample size. It was possible that in our study baseline cortisol profiles were relatively “normal” in the cancer survivors, unlike the flatter profiles characteristic of cancer patients undergoing treatment or with metastatic cancer (Carlson et al., 2007a). Finally, since we did not collect the Waking sample the same day as the other four samples, we could not assess intervention effects on the cortisol awakening response (CAR) or the sAA awakening response. The CAR has been commonly used as an important measure to provide

understanding about mechanisms of regulation of stress hormone systems and circadian rhythms, as well as insight into clinically-relevant conditions (Clow et al., 2010).

The present study was an exploratory study, and thus it has certain limitations, including small sample sizes, collecting saliva samples over the course of a single day rather than two to three consecutive days, and not collecting the Waking sample on the same day as the other saliva samples collected later in the day.

The results of our main study in cancer survivors with sleep disturbance demonstrated that MBB and MM were more efficacious in improving sleep compared with that of the SHE group, and MBB was more efficacious in increasing mindfulness, well-being, and self-compassion, and in reducing depressive symptoms compared with that of SHE (manuscript under review). Future studies will more comprehensively evaluate the effects of mind-body interventions in regulating both physiological and psychological variables, to provide a more integrative understanding of mechanisms whereby these mind-body interventions exert their effects.

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Figure 1.

Baseline mean (and SEM) levels of sAA (closed circles) and salivary cortisol (closed squares) depicting changes in biomarker levels across a 24-hr period, comprising four collections on Day 1, Post-awake (30 min after awakening), Noon, Afternoon (5 pm), Evening (before bedtime, ~10 pm) and the fifth sample collected upon awakening (Waking) on the following day, Day 2, as separated by the vertical dotted line (see main text for further information). The data represent baseline (PRE) levels for all 57 participants.

a.

b.

Figure 2a and b.

Waking (a) sAA and (b) salivary cortisol levels showing sample means at PRE and POST (solid circles) and their standard errors (vertical bars) relative to the change expected in the absence of treatment effects (empty circles and dashed lines). The observed changes include the biasing effects of regression to the mean and chance baseline differences resulting from randomization. Arrows show magnitude and direction of systematic change corrected for this bias, and correspond to the estimated group treatment differences in the statistical analysis. Horizontal dashed line is overall observed mean at PRE. Figure shows raw values; statistical tests were based on log-transformed values to reduce skew and meet normal theory test assumptions. In Figure 2a, the mean Waking sAA level in MBB at POST was lower than that in SHE ($p=.019$, test of equal MBB and SHE treatment effect, see text).

Figure 3.

MOS-SS Sleep Problems Index II scores showing sample means at PRE and POST (solid circles) and their standard errors (vertical bars) relative to the change expected in the absence of treatment effects (empty circles and dashed lines). The observed changes include the biasing effects of regression to the mean and chance baseline differences resulting from randomization. Arrows show magnitude and direction of systematic change corrected for this bias, and correspond to the estimated group treatment differences in the statistical analysis. Horizontal dashed line is overall observed mean at PRE. While all interventions indicated significant reductions in sleep problems at POST, MBB was lower than SHE ($p=.001$, test of equal MBB and SHE treatment effect, see text).

Table 1
Cancer Survivor Demographics and Clinical Characteristics for Participants in each Intervention Group Reported at Baseline

	SHE	MBB	MM
Age (yr.)	51.6 (10.7) *	55.4 (9.6)	50.8 (9.10)
Females	14	13	16
Males	4	6	4
Clinical Characteristics			
Previous diagnosis of:			
Breast cancer	9	8	14
Other cancers #	10 ^	13 ^	6
First cancer diagnosis (median years)	4 yrs, 2 mos	3 yrs 8 mos	2 yrs 10 mos
Insomnia diagnosis	6	8	8
Clinical depression	6	6	7
Self-reported medications/supplements:			
Sleep medications	5	4	5
Antidepressants	9	7	9
Any prescription medications	18	15	19
Any supplements/herbal remedies	16	17	17

* SD in parentheses

type of cancer, including ovarian, endometrial, testicular, prostate, lung, melanoma, ependymoma, leukemia, kidney, lymphomas (Non-Hodgkins, CNS), skin carcinoma, brain, thyroid, peritoneal

^ certain individuals reported more than one cancer site.

Table 2
Raw Means (and SDs) of sAA and Cortisol for, a. Waking sample, b. Daily output (AUCi),
and c. for Sleep scores, across the three interventions

	Period	SHE	MBB	MM
a. Waking Sample				
sAA (U/ml)	PRE	29.77 (58.44)	49.60 (40.62)	45.64 (49.12)
	POST	39.41 (58.28)	22.45 (16.03) [#]	44.71 (60.23)
Cortisol (nmol/L)	PRE	14.26 (8.14)	17.17 (23.05)	13.88 (9.37)
	POST	10.84 (5.87) [*]	14.02 (6.98)	14.15 (9.01)
b. AUCi				
sAA	PRE	2.05 (6.08)	3.11 (5.31)	4.03 (4.14)
	POST	2.97 (3.61)	3.59 (7.61)	3.45 (3.28)
Cortisol	PRE	-6.12 (3.34)	-4.75 (4.71)	-6.21 (2.96)
	POST	-5.91 (3.62)	-6.52 (2.80)	-5.76 (5.35)
c. Sleep Scale				
Sleep Problems Index-II	PRE	54.94 (18.31)	58.01 (14.64)	63.33 (12.70)
	POST	47.22 (16.21) ^{**}	32.13 (14.81) ^{***}	43.64 (17.44) ^{***}

[#]
p=.010

^{*}
p=.052

^{**}
p<.01

^{***}
p<.001 (compared with baseline levels within each Intervention, respectively)

Table 3
Raw Means (and SDs) of Diurnal profiles for sAA and Cortisol across the three Interventions

Intervention	Collection Time	sAA (U/ml)		Cortisol (nmol/L)	
		PRE (baseline)	POST	PRE (baseline)	POST
SHE	Post Awake	24.22 (29.24)	22.43 (20.73)	12.08 (10.06)	12.77 (9.57)
	Noon	36.13 (46.23)	43.17 (45.79)	7.96 (8.45)	6.57 (3.86)
	Afternoon	38.67 (40.96)	51.75 (52.42)	4.58 (7.04)	3.00 (1.95)
	Evening	41.38 (60.74)	32.78 (34.00)	3.05 (5.79)	2.18 (1.90)
MBB	Post Awake	30.20 (21.68)	40.13 (67.11)	14.53 (6.68)	14.69 (7.09)
	Noon	53.66 (39.59)	68.24 (77.33)	9.38 (5.92)	7.65 (6.34)
	Afternoon	55.15 (36.67)	42.99 (32.44)	5.41 (2.57)	4.78 (5.45)
	Evening	50.14 (64.91)	53.35 (44.34)	3.20 (4.38)	2.28 (1.02)
MM	Post Awake	34.56 (41.20)	43.48 (67.45)	14.87 (6.77)	12.70 (7.68)
	Noon	83.89 (76.94)	80.55 (75.79)	7.85 (4.31)	7.14 (3.53)
	Afternoon	70.70 (70.70)	87.73 (113.60)	4.50 (3.20)	3.53 (2.07)
	Evening	50.02 (43.66)	69.12 (87.36)	4.81 (8.88)	1.72 (1.29)