An examination of subjective and physiological stress-related factors in breast cancer survivors

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Abstract

Dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis activity has been commonly observed among breast cancer patients and has been linked to adverse health consequences. However, whether these alterations persist long after the cancer diagnosis has not been well-documented.

In the first study, the diurnal cortisol rhythms and the cortisol stress response of breast cancer survivors who had completed all local and/or systemic adjuvant therapy with the exception of hormonal therapy were compared to those of women without a history of cancer. The Trier Social Stress Test was used to elicit a moderate stress response and the subjective levels of stress of participants were recorded using visual analog scales. The results indicate similar diurnal patterns in both groups; however, significant differences in stress reactivity were noted, with breast cancer survivors displaying a relatively flat profile following the acute stress induction. Subjective levels of psychological stress were similar in both groups, indicating that the subjective appraisal did not account for the blunted cortisol stress response.

In the second study, the impact of the stressful life events that happened during the previous year on the cortisol stress response was analyzed in the same groups of participants. The frequency of stressful life events as well as their subjective impact was documented using the Life Experience Survey. Results suggest no group differences between the total number of stressful life events and their perceived effect. However, the number of stressful life events and their perceived impact correlated negatively with the peak cortisol concentration in breast cancer survivors. The results suggest that the cumulative effect of stressful life events contribute significantly to the low levels of cortisol reported in breast cancer survivors following a stressful situation.

Together, these studies emphasize that breast cancer survivors are at risk of presenting a subtle alteration of their HPA axis activity when their system is challenged and that an accumulation of stressors plays a role in this dysregulation. These results reinforce the need for interventions intended to reduce the levels of psychological stress experienced by breast cancer survivors.

Résumé

Une altération du fonctionnement de l'Axe hypothalamo-hypophyso-surrénalien (HHS) a été communément rapporté chez les survivantes du cancer du sein et est lié à des conséquences néfastes pour la santé. Toutefois, il n'a pas été clairement établi que cette dérégulation persisterait longtemps après un diagnostic de cancer.

Dans la première étude, les rythmes diurnes de sécrétion de cortisol de même que suivant un stresseur aigu d'un groupe de survivantes du cancer du sein ayant complété tous leurs traitements locaux et/ou adjuvant à l'exception du traitement hormonal ont été comparés à ceux d'un groupe de femme sans antécédent de cancer. Le protocole *Trier Social Stress Test* a été utilisé pour susciter une réponse de stress modérée et les niveaux de stress subjectifs des participantes ont été consignés à l'aide d'échelles visuelles analogues. Les résultats ont indiqué des profils circadiens de cortisol similaires chez les deux groupes. Toutefois, la réponse de stress des survivantes du cancer du sein s'est avérée significativement plus faible que celle des femmes sans antécédents de cancer. Les niveaux de stress subjectifs se sont avérés similaires pour les deux groupes, suggérant que l'évaluation subjective du stresseur n'était pas responsable de la réponse hormonale atténuée.

Dans la seconde étude, l'impact cumulatif d'évènements stressants survenus au cours de la dernière année sur la réponse de stress a été évalué chez les deux groupes de participantes. Le nombre d'évènements stressants de même que leurs évaluations subjectives ont été répertoriés à l'aide de l'échelle *Life Experience Survey*. Aucune différence significative n'a été notée entre le nombre d'évènements stressants et leurs évaluations subjectives chez les deux groupes de participantes. Toutefois, le nombre de stresseurs et leurs évaluations subjectives ont corrélé négativement avec l'apogée de la concentration de cortisol des survivantes du cancer du sein. Les résultats suggèrent que l'effet cumulatif des

stresseurs récents contribuent de façon significative au niveau réduit de cortisol noté chez les survivantes de cancer à la suite d'un évènement stressant.

Ensemble, ces études suggèrent que les survivantes du cancer du sein sont à risque de présenter une légère altération du fonctionnement de l'axe HHS en période de stress et que l'accumulation de stresseurs joue un rôle dans cette dérégulation. Ces résultats renforcent le besoin d'interventions visant à réduire le niveau de stress psychologiques vécu par les survivantes du cancer du sein.

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Adrenocorticotropic hormone	ACTH
Analysis of variance	ANOVA
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Bidimensional Fatigue Scale	BFS
Corticotropin-releasing hormone	CRH
Hypothalamo-pituitary-adrenal	HPA
Life Experience Survey	LES
Paraventricular nucleus	PVN
Social Readjustment Rating Scale	SRRS
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Statistical Package for the Social Sciences	SPSS
Suprachiasmatic nucleus	SCN
Sympathetic nervous system	SNS
Trier Social Stress Test	TSST
Visual Analog Scale	VAS

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General Introduction

Breast cancer is one the most common forms of cancer diagnosed in women in Canada (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). In 2014, roughly 24,400 Canadian women received a diagnosis of breast cancer and 5000 succumbed to it (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). According to the same source, one in nine Canadian women will develop breast cancer in her lifetime.

On a more positive note, advances in the fields of screening and treatment have led to a decline in the mortality rate of breast cancer patients (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). The incidence of breast cancer has been stable since the mid-1980s and the mortality rate has been continuously diminishing since 1986, with a decline rate of 2.4% since 2000 (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). Indeed, it is suggested that 88% of women diagnosed with breast cancer survive more than five years following the initial cancer diagnosis, while 82% of breast cancer survivors are expected to survive more than 10 years (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). Thus, women diagnosed with breast cancer represent one of the largest groups of cancer survivors in Canada (Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). While the increase in survivor rates is encouraging, these results emphasize even more the importance of research related to the psychological and physical well-being of breast cancer survivors later on in their lives. One important factor that has been shown to influence both psychological and physical health is the prevalence of higher levels of psychological stress in that population (Deimling, Bowman, Sterns, Wagner, & Kahana, 2006; Liao, Chen, Chen, & Chen, 2008; Lim, Devi, & Ang, 2011; Mehnert & Koch, 2007; Montgomery & McCrone, 2010).

Stress and anxiety along the breast cancer trajectory

A diagnosis of breast cancer is often perceived as the beginning of a long process that includes surgical procedures, chemotherapy and radiotherapy. If the body is confronted with difficult challenges, so is the psyche. From the first signs and symptoms suggesting a serious disease, to the cancer diagnosis itself, followed by a series of heavy treatments and side effects, the development of cancer carries a number of difficult and stressful transitions. Psychosocial stressors associated with cancer include the fear of death but also anticipated adverse changes in social and vocational roles as well as concerns regarding long-term physical and psychological sequelae (Lebel, Zosberg, Edgar, & Devins, 2007; Liu et al., 2011; Tiedtke, de Rijk, Dierckx, de Carterlé, Christiaens, & Donceel, 2010). Women diagnosed with cancer are also faced with making challenging decisions and may experience a sense of loss of control over the course of their disease (Sharpley & Christie, 2007). Concerns regarding the negative impact of cancer on loved ones is also a common burden experienced by breast cancer survivors (Liu et al., 2011). Once the cancer has been fought and the woman is considered disease free, the stress and anxiety can persist under the form of a fear of cancer recurrence. Fear of cancer recurrence can be defined as a fear that the cancer will reoccur or progress at the same or different location in the body (Vickberg, 2003).

Thus, a large body of literature suggests that women diagnosed with breast cancer usually present high levels of stress and anxiety at the beginning of their disease and that this distress may persist several years after diagnosis. For example, in a study by Liao and colleagues (2008), levels of anxiety were recorded in women undergoing the diagnostic phase for suspected breast cancer using the State Anxiety Inventory (Spielberger, Gorusuch, Lushene, Vagg, & Jacobs, 1983). Severe average levels of anxiety were reported at three time points: upon the notice of

further breast biopsy, while the patient was waiting for the biopsy procedure and immediately following the physician's explanation of the biopsy result. Higher levels of anxiety were also reported in women that were diagnosed with a malignant tumor. Furthermore, clinical levels of distress were reported in 41% of breast cancer survivors prior to their consultation regarding their breast cancer surgery (Hegel et al., 2006), while, immediately before their breast cancer surgery, significant levels of anxiety were reported in 75% of women (Aviado-Langer, 2014). Along the same lines, in another study by Mehnert and Koch (2007), levels of anxiety were assessed in breast cancer patients who were recovering from their first breast cancer surgery and a re-test was also completed six months post-surgery. Using the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983), significant levels of anxiety were noted in 39.6% of breast cancer survivors following the surgery, while 32.7% of breast cancer survivors still reported high levels of anxiety six months post-surgery. Results of this study suggest that even though the overall proportion of women experiencing high levels of anxiety diminishes over time, a significant portion of women still manifest significant distress six months after the surgery.

Anxiety has also been assessed in long-term breast cancer survivors (Deimling et al., 2006; Mehnert & Koch, 2008). For example, in a study by Mehnert and Koch (2008), 38% of breast cancer survivors who were on average four years post-diagnosis (ranged from 1.5 to 6.5 post-diagnosis) reported moderate to high levels of anxiety and 12% reported symptoms of post-traumatic stress disorder. In addition, in breast cancer survivors being on average ten years post-diagnosis (ranged from 5-34 years post-diagnosis), approximately one third of the sample reported worries about a recurrence, worries about a second cancer occurrence, as well as worries that the symptoms they experience may be from cancer (Deimling, et al., 2006).

Altogether, these studies suggest that breast cancer survivors are confronted with high levels of anxiety early on in their breast cancer trajectory and that this distress may persist several years after the end of treatments. Thus, the experience of breast cancer may be considered a situation of chronic stress. While a punctual period of stress can be beneficial to the organism, chronic stress may adversely interfere with the normative activity of the stress system.

The history of the concept of stress and the physiological stress system

The articulation of the concept of stress is generally attributed to the pioneer work of Walter B. Cannon (1915; 1967) and Hans Selye (1978). Cannon originally envisaged stress as part of the larger notion of *homeostasis*, a term that he coined, suggesting that the organism tends to maintain itself in a state of equilibrium despite constant internal and external changes (Cannon, 1967). In that context, Cannon considered stress as any disturbance of the organism's homeostasis (Levine, 2005). He centered his research on the type of stimuli needed to activate the stress response (including some psychological stressors) as well as the physiological mechanisms responsible for the detection of changes in the external environment and the activation of the proper compensatory mechanisms (Lovallo, 2005). One of the biggest differences between Cannon's and Selye's comprehension of stress is that while Cannon appreciated it in terms of the stimulus required to elicit the stress response, Selye's conceptualisation used stress to denote the response itself (Levine, 2005). Indeed, Selye (1978) defined stress as: "the nonspecific response of the body to any demand". Seyle was interested in unveiling the sets of responses orchestrated by the body to deal with environmental demands, or, as he labelled, *stressors*. However, he limited his research to stressors of physical nature such as pain, cold, heat, as well as extraction of ovaries, kidneys, and other organs, while ignoring the psychological aspects of stress (van Praag, de Kloet, & van Os, 2004). His other area of interest

was related to the long term activation of the stress response and, in that sense, he developed a paradigm that he called the *General Adaptation Syndrome*. In his model, he postulated that the persistent activation of the stress response would encompass three different stages eventually leading to exhaustion and physiological harm (van Praag et al., 2004; Lovallo, 2005). Thus, Seyle's groundwork highlighted the idea that while the stress response is overall adaptive, its hyperactivation can also threaten the organism over time. Taken together, while Cannon's and Selye's *avant-garde* work may have failed to agree on the exact definition of stress, their work nonetheless established that there was a physiological basis associated with the stress response and, in that sense, both scientists recognised the role of the adrenal gland (Cannon, 1915; Selye, 1978).

Other leading scientists, such as John Wayne Masson, complemented the investigations of Cannon and Seyle by emphasising that psychological variables such as emotional arousal played an important role in the human stress response and could be seen as linking the stressor to the stress reaction (Mason, 1971). Masson was particularly interested in studying the kind of psychological factors that reliably induced a stress response in humans. Specifically, he found that situations that were novel, unpredictable, threatening to the ego, and uncontrollable were the most susceptible to create a stress response (Mason, 1968).

Later on, Lazarus and Folkman (1984) further emphasized the active role of the individual in the stress experience via the subjective cognitive appraisal. In their book, *Stress, appraisal and coping*, they indicated that the *primary appraisal* consisted of an evaluation of the level of dangerousness of a situation or stimulus while the *secondary appraisal* entailed the individual's appraisal of his or her own ability to meet the challenge, in other words, to cope with the situation. They also argued that the conceptualisation of stress as either a stimulus or a

response was an incomplete illustration of the concept and that the dilemma that was occurring fell into the typical psychological tradition of understanding human behaviour under simplistic stimulus-response paradigm. In that sense, they stated that the definition of stress in terms of a stimulus creating a stress response would be inadequate because it would not account for individual differences in the evaluation of the event. They further argued that a response could not reliably be judged as a psychological stress reaction without reference to a stimulus and a stimulus could not be interpreted as stressful if no stress reaction occurred. Thus, one of the hallmarks of their work lies in the idea that stress is defined by the *stimulus-response relationship*.

Taken together, the groundwork developed over the years by Cannon, Seyle, Mason, Lazarus, and Folkman are of particular interest in the comprehension of the long term effect of cancer on the stress system of breast cancer survivors. While the experience of cancer can been seen as a major source of psychological stress, it also implicates other physical stressors such as the tumor itself as well as surgery and treatments, stressors that can also contribute to the activation of the stress system. Thus, both psychological stressors and physical stressors should be taken into account while studying the effect of cancer on the physiological stress system of breast cancer survivors.

The Hypothalamo-pituitary-adrenal axis

The hypothalamo-pituitary-adrenal (HPA) axis can be defined as a set of interactions between the hypothalamus, the pituitary gland, and the adrenal cortex (Nicolson, 2008). The HPA axis has been described as one of the main physiological system dedicated to buffer the effect of stress on the body in order to maintain homeostasis (McEwens, 1998; McEwen & Wingfield, 2003; Peters & McEwens, 2012). The hormone cortisol, secreted by the HPA axis,

also plays a number of regulatory functions in humans such as glucose production, fat metabolism, and immune function (Ice, Katz-Stein, Himes, & Kane, 2004; Stone et al., 2001). The HPA axis has been first described in a body of emerging literature in the 1960s (Nicolson, 2008). Over the last decades, the number of studies devoted to its better understanding has flourished given the increasing ease in which its activity could be measured as well as its association with health and disease (Nicolson, 2008). Over the years, the "end" product of the HPA axis, the hormone cortisol, has been shown to follow a baseline circadian rhythm as well a set of predictable steps designed to respond to an acute stressful situation (Nicolson, 2008).

The secretion of cortisol

Analogous to most of the human hormones, cortisol is not secreted in a continuous fashion, but instead in a series of pulsatile episodes that occur throughout the day and night (Kirschbaum & Hellhammer, 1989; Young, Abelson, & Lightman, 2004). In individuals in good physical and psychological health, 10 to 20 pulsations of cortisol occur over the course of a 24 hour period, with intermittent pulsations following approximately at one to two hour intervals (Ice, et al., 2001; van Pragg, et al., 2004). The frequency and amplitude of the pulsatile episodes of cortisol varies according to the circadian rhythms as well as the stress response (van Praag et al., 2004). The secretion of cortisol into the systemic circulation is orchestrated mainly by the hypothalamus which receives and integrates information from many sources. The hypothalamus initiates the synthetization and release of two neurohormones, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN). These messengers are then released into the portal vessel to the anterior pituitary gland where they act synergistically to activate the secretion of the adrenocorticotropic hormone (ACTH) which reaches the adrenal cortex and elicits the release of glucocorticoids, a class of steroid hormones

that includes cortisol (Buckingham, 2000; Herman, 2011; Thiel & Dretsch, 2011). Once cortisol is released into the general circulation, it gradually inhibits the release of CRH and AVP, creating a negative feedback loop that resets the system to its initial state (Buckingham, 2000; Herman, 2011; Thiel & Dretsch, 2011). This sophisticated cascade of reactions has been shown to lead to robust cortisol diurnal rhythms as well as a predictable cortisol stress response in healthy individuals (Buckingham, 2000).

The typical diurnal rhythm. Higher pulsatile amplitudes of cortisol are recorded in the early morning and smaller amplitudes in the evening and night, resulting in a cortisol circadian rhythm (Kirschbaum & Hellhammer, 1989; Young, et al., 2004). The typical diurnal cortisol pattern is characterized by a 50-100% increase of cortisol 30 to 45 minutes following awakening as well as a diminution of cortisol throughout the day with the lowest levels observed around midnight (Clow, Hucklebridge, & Thorn, 2010; Kirschbaum & Hellhammer, 1989; Kirschbaum & Hellhammer, 2000; Stone et al., 2001). Thereafter, cortisol gradually increases at around 2:00AM (Ice, et al., 2004). The circadian rhythms are believed to help the organism to continuously adapt to the environment (Strahler, Berndt, Kirchbaum, & Rohleder, 2010). For example, it has been hypothesized that peak levels of cortisol in the early morning support the energy available for the organism at the beginning of the day and increase the appetite for carbohydrates (Daly, Delaney, Doran, MacLachlan, 2011; Smyth et al., 1997). Likewise, the decline of cortisol in the late afternoon and evening has been suggested to be a way for the organism to recover at the end of the day (Miller, 2007).

At the anatomical level, similar to most of the endogenous substances of the organism, the cortisol circadian rhythm is influence by the hypothalamic suprachiasmatic nucleus (SCN; Clow, et al., 2010; Figueiro & Rea, 2010; Nater, Rohleder, Scholtz, Ehlert, & Kirschbaum,

2007). The SCN is directly connected to the paraventricular nucleus of the hypothalamus via direct hypothalamic neural connections (Mazzoccoli, Giuliani, & Sotherm, 2012). The SCN integrates both endogenous and exogenous information to induce the rhythmic release of cortisol. For example, diurnal cortisol circadian rhythm has been shown to be influenced by light exposure, sleep-wake cycle, sex, age, napping, meals (Smyth et al., 1997; Ice et al., 2004; Strahler, Berndt, et al., 2010) as well as stressful experiences (Dettenborn, James, van Berge-Landry, Valdimarsdottir, Montgomery, & Bovbjerg, 2005), and breast cancer (Abercrombie, Giese-Davis, Sephton, Epel, Turner-Cobb, & Spiegel, 2004; Alexander, Minton, & Andrews, & Stone, 2009; Bower, Ganz, Dickerson, et al., 2005; Carlson, Campbell, Garland, & Gossman, 2007; Dedert et al., 2012; Giese-Davis, Sephton, Abercrombie, Duran, & Spiegel, 2004; Giese-Davis, DiMiceli, Sephton, & Spiegel, 2006; Ho, Fong, Chan, & Chan, 2013; Sephton, Sapolsky, Kraemer, & Spiegel, 2000; Touitou, Bogdan, Lévi, Benavides, & Auzéby, 1996; Touitou, Lévi, Bogdan, Benavides, Bailleul, & Misset, 1995; Vedhara, Tuinstra, Miles, Sanderman, & Ranchor, 2006).

The typical stress response. The cortisol secretion pattern following an acute stressor have been well-studied over the last decades (Foley & Kirschbaum, 2010; Kudielka, Hellhammer, Wust, 2009; Kudielka & Wust, 2010). Unlike other endocrine and neuronal response circuits, the HPA axis stress response takes several minutes before signals are observed in the periphery (Kirschbaum & Hellhammer, 2000). While ACTH levels begins to rise within five minutes following the onset of a stressor, the cortisol increases are observed five to 20 minutes with peak levels occurring 10 to 30 minutes following stressor cessation (Kirschbaum & Hellhammer, 2000). The increase in cortisol levels have been shown to vary depending on the stressor severity; for example, using the Trier Social Stress Test (TSST), a laboratory protocol

designed to elicit a moderate stress response, a two to three-fold elevation of cortisol compared to baseline levels have been noted in a majority of participants (Kudielka. Hellhammer, & Kirschbaum, 2007). Furthermore, even though cortisol initiates a strong negative feedback loop, its continuous secretion over several hours have been observed in prolonged stressful situations (Kirschbaum & Hellhammer, 2000). For instance, a constant increase of cortisol has been noted for more than four hours in a sample of marathon runners, with average peak levels occurring around 30 minutes following the end of the race (Kirschbaum & Hellhammer, 2000). Finally, similar to diurnal rhythms, a history of breast cancer can also lead to alteration of the typical cortisol stress reaction (Bower, Ganz, & Aziz, 2005; Palesh et al., 2008; van der Pompe, & al., 1996).

Breast cancer and cortisol rhythms

The assessment of cortisol secretion rhythms in breast cancer survivors is a growing topic in the scientific literature. Cortisol activity has been evaluated at various stages of the disease including recently diagnosed breast cancer patients (Vedhara, et al., 2006), early stage breast cancer patients (Dedert et al., 2012, Ho, et al., 2013), metastatic breast cancer patients (Abercrombie, et al., 2004; Giese-Davis et al., 2006; Giese-Davis, DiMiceli, Sephton, & Spiegel, 2006; Giese-Davis, et al., 2004; Palesh et al., 2008; Sephton, et al., 2000; Spiegel, Giese-Davis, Taylor, & Kraemer, 2006; Touitou, et al., 1995; Touitou, et al., 1996; Turner-Cobb, Sephton, Koopman, Blake-Mortimer, & Spiegel, 2000; van der Pompe, Antoni, & Heijnen, 1996) and breast cancer survivors who are considered disease free (Alexander, et al., 2009; Bower, Ganz, & Aziz, 2005; Bower, Ganz, Dickerson, Petersen, Aziz, & Fahey, 2005; Carlson, et al., 2007; Porter, Mishel, Neelon, Belyea, Pisano, & Scott Soo, 2003). Some studies have focused on the diurnal cortisol rhythms (Abercrombie, et al., 2004; Alexander, et al., 2009;

Bower, Ganz, Dickerson, et al., 2005; Carlson, et al., 2007 ; Dedert et al., 2012; Giese-Davis, et al., 2004; Giese-Davis, DiMiceli, et al., 2006; Ho, et al., 2013; Sephton et al., 2000; Touitou et al., 1995; Touitou et al., 1996; Vedhara, & al., 2006), others analysed the cortisol stress response (Andreano, Waisman, Donley, & Cahill, 2012; Bower, Ganz, & Aziz, 2005; Palesh et al., 2008; van der Pompe, & al., 1996), while some included both the diurnal rhythms and the acute stress patterns (Giese-Davis, Wilhem, et al., 2006; Porter, et al., 2003; Spiegel, et al., 2006).

Diurnal cortisol rhythms in breast cancer survivors

Metastatic breast cancer patients. The majority of the studies that focus on cortisol activity in breast cancer survivors have focused on advanced forms of the disease such as metastatic breast cancer. Metastatic cancer can be defined as a cancer that has spread from its site of origin to another place in the body, the most common sites of cancer metastasis being the lungs, bones, and liver (National cancer institute, 2011). Metastatic breast cancer requires complex treatments such as systemic therapy (chemotherapy, biological therapy, targeted therapy, hormonal therapy), local therapy (surgery, radiation therapy), or a combination of these treatments (National Cancer Institute, 2011). As such, it is not surprising to see that most metastatic breast cancer patients present significant alteration of their cortisol diurnal rhythms (Abercrombie et al., 2004; Sephton et al., 2000; Touitou, et al., 1995; Touitou et al., 1996). In their study, Touitou and his collaborators (1996) found changes characterized by sustained high levels of cortisol over a 24 hour period and/or erratic peaks and troughs and/or flattened rhythms. Furthermore, in a study by the same research group, no visible rhythmic patterns of cortisol were discerned over a 48-h period in breast cancer patients with liver metastases (Touitou et al., 1995). Similarly, in Sephton and colleagues' study (2000), 49% of the sample of metastatic breast cancer patients had peak concentrations that occurred later in the day than is typical and 14% had

no apparent peak (flatter slope). Finally, in the samples reported by Abercrombie and collaborators (2004), metastatic breast cancer patients had significantly flatter diurnal cortisol slopes compared to that of women in the control group.

Other studies have also emphasised that psychological variables such as depression (Giese-Davis, Wilhelm, et al., 2006), emotional repression (Giese-Davis et al., 2004; Giese-Davis, DiMiceli, et al., 2006), high anxiety (Giese-Davis et al., 2004), sleep quality (Palesh et al., 2008) as well as lack of social support (Turner-Cobb, 2000) also contribute to the alteration of the diurnal cortisol rhythms seen in metastatic breast cancer patients.

Early-stage breast cancer patients. On a more positive view, in patients diagnosed with less severe cancer stage, patterns of diurnal cortisol rhythms appear to be typical (Dedert et al., 2012; Ho et al., 2013; Vedhara et al., 2006). In Vedhara and collaborators' study (2006), no significant differences in cortisol concentrations at any time points during the day were noted between breast cancer patients and women without a history of cancer. Although the cancer stage was not specified in the study, it is possible that the research participants had less advanced breast cancer given that a survival prognosis of at least 15 months was one of the inclusion criteria. Furthermore, in Dedert and colleagues' study (2012), only breast cancer patients that showed high psychological distress, avoidant coping and disruptive rest/activity cycles presented with flattened diurnal cortisol rhythms while the rest of the sample showed circadian patterns that were typical. Similarly, in Ho and collaborators' study (2013), breast cancer patients with low perceived health and social support as well as poor sleep quality presented with slightly dysregulated cortisol slopes while the rest of the breast cancer sample showed diurnal rhythms that were comparable to the norm. Thus, it is possible that disease severity and invasive treatments play an important role in the significant dysregulation of cortisol diurnal rhythms seen

in metastatic breast cancer patients since no such alterations were visible in early-stage breast cancer patients in good psychological health. However, similar to metastatic patients, other variables such as psychological distress, coping, social support and sleep may also play a role in the subtle dysregulation of diurnal cortisol rhythms seen in some early-staged breast cancer patients.

Long-term breast cancer survivors. Finally, some authors have investigated the diurnal cortisol rhythms of breast cancer survivors considered disease free (Alexander et al., 2009; Bower, Ganz, Dickerson, et al., 2005; Carlson, et al., 2007; Porter, et al., 2003). Importantly, all four studies included breast cancer survivors originally diagnosed with non-metastatic breast cancer. In Carlson and collaborators' study (2007), breast cancer survivors who had finished their primary treatment at least three months earlier showed typical diurnal cortisol rhythms that were not significantly different from that of women in the control group. However, divergent results were found in another study entailing breast cancer survivors being three to five years posttreatment (Porter et al., 2003). In their study, the mean levels of cortisol in breast cancer survivors over three typical days were significantly higher than those of women in the control group. However, the authors reported that a history of specific medical variables such as higher cancer stage, mastectomy, and chemotherapy contributed significantly to the atypical diurnal cortisol levels seen in breast cancer survivors. Furthermore, the researchers also assessed the levels of cortisol on the days surrounding a routine mammogram and found that breast cancer survivors reacted with lower levels of cortisol, while women in the control group reacted with elevated levels of cortisol, suggesting an association between higher levels of psychological stress and lower levels of diurnal cortisol in breast cancer survivors. In Bower, Ganz, Dickerson and collaborators' study (2005), higher levels of cortisol concentration in the evening were only

present in fatigued breast cancer survivors, while non-fatigued breast cancer survivors showed typical diurnal cortisol rhythms. However, these results were not replicated in a study by Alexander and collaborators (2009) who found no significant differences between the urinary cortisol levels of breast cancer survivors with and without fatigue.

These results suggest that breast cancer survivors considered disease free seem to present, for the most part, typical cortisol diurnal rhythms. However, important medical variables such as cancer stage, treatment, and fatigue have been raised as factors that can alter the typical cortisol rhythms of long-term breast cancer survivors.

Reactive cortisol in breast cancer survivors

The cortisol stress response of breast cancer survivors has been assessed via the use of laboratory protocols design to induce a moderate stress response, such as the TSST (Bower, Ganz, & Aziz, 2005; Spiegel et al., 2006), the cold-pressure test (Andreano et al., 2012), and other social-evaluative designs (van der Pompe et al., 1996). An attenuation of cortisol as well as a flat cortisol profile were noted in both studies of metastatic breast cancer patients (Spiegel et al., 2006; van der Pompe et al., 1996). However, in non-metastatic breast cancer patients, only fatigued and breast cancer survivors treated with GnRH agonist Lupron displayed a blunted cortisol stress response (Andreano et al., 2012; Bower, Ganz, & Aziz, 2005). In the study by van der Pompe and colleagues (1996), an atypical decrease in cortisol concentration was noted in metastatic breast cancer patients following their participation in a stressful social-evaluative protocol. The protocol required research participants to pretend, for a duration of four minutes while being videotaped, that they were accused of stealing in a shop. Similarly, Spiegel and collaborators (2006) found that metastatic breast cancer survivors manifested a significantly flatter cortisol stress response compared to that of women without a history of cancer following

their participation in the TSST (Spiegel et al., 2006). However, in a study by Bower, Ganz and Aziz (2005), only fatigued breast cancer survivors reported an attenuated cortisol stress response while non-fatigued breast cancer survivors presented the typical response pattern. Likewise, Andreano and colleagues (2012) found that breast cancer survivors treated with the GnRH agonist Lupron, a type of hormonal therapy, presented with a blunted cortisol stress response while breast cancer survivors who were not treated with Lupron manifested the typical cortisol stress response.

In conclusion, atypical patterns of diurnal and reactive cortisol secretion seem to be the hallmark of metastatic breast cancer patients. However, in early-stage breast cancer patients and long-term breast cancer survivors, the diurnal pattern seems to be fairly robust. In contrast, dysregulation of the cortisol stress response appears to be more frequent in early-stage and long-term breast cancer survivors. Moreover, medical variables such as cancer stage, cancer treatment, time since diagnosis, fatigue and sleep quality seem to play a role in their reactive cortisol response. Other psychological variables have also been highlighted as potential factors in the dysregulation of reactive cortisol such as psychological distress, depression, anxiety, and social support.

The normative activity of the circadian rhythm as well as the stress response of cortisol have been associated with health and survival while its dysregulation has been linked to pathology (McEwens, 1998; McEwen & Wingfield, 2003; Peters & McEwens, 2012). Furthermore, in breast cancer patients, flatter diurnal cortisol rhythms have been linked to disease severity (Abercrombie et al., 2004) and have been shown to predict shorter subsequent survival times (Sephton et al., 2000). Similarly, atypical cortisol diurnal rhythms have been associated with cancer progression (Touitou, et al., 1995). Thus, documenting if long-term breast

cancer survivors present alteration of their cortisol secretion patterns and understanding which factors contribute to its dysregulation is of great importance in the context of health prevention and quality of life following a history of breast cancer. One of the factors that has been associated with alterations of both the circadian rhythms and the stress response in multiple studies is chronic stress. As well, prolonged period of psychological stress and anxiety have been associated with the experience of breast cancer. The accumulation of stressors is thus an important factor to consider in the understanding of the effect of the experience of cancer on the physiological stress system of breast cancer survivors.

The cumulative effect of stress and the Hypothalamo-pituitary-adrenal axis

The HPA axis fluctuates within broad ranges in order to buffer the effect of stress on the homeostatic systems that need more stability such as body temperature and blood composition (McEwens, 1998; 2003). However, under a situation of chronic stress, the constant adaptation of the HPA axis activity can lead to its dysregulation. Selye (1978) was one of the first authors to acknowledge the paradox that while the stress response is overall adaptive, it can also threaten the organism via hyperactivation of the adrenal gland. Following his pioneer work, hyperactivation of the HPA axis and secretion of high levels of cortisol was generally assumed in a situation of chronic stress. Numerous studies abounded in that vein and the phenomenon of hypercortisolism has been reported in both cortisol diurnal rhythms (Dettenborn, et al., 2005) and cortisol stress reactivity (Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003; Gonzalez-Cabrera, Fernandez-Prada, Iribar-Ibabe, & Peinado, 2014; Heim, et al., 2000; Laufer, Ansermet, von der Weid, Popovic, Torrisi, & Pierrehumbert, 2012; van der Hal-Van Raalte, et al., 2008) of chronically stressed individuals. However, in the last decades, the phenomenon of hypocortisolism has been reported as well in individuals who have undergone long-term

exposure to psychological stress and early life trauma (Bergen, et al., 2012; Carpenter, Shattuck, Tyrka, Geraciotti, & Price, 2011; Juster, et al., 2011; Gordis, Granger, Susman, & Trickett, Gordis, Peckins, & Susman, 2014; Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Macmillan, et al., 2009; Matthews, Gump, & Owens, 2001; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004; Trickett, et al., 2014). In light of these conflicting results, it has been suggested that hypocortisolism would occur following a prolonged period of excessive cortisol secretion as a way for the body to compensate for long-term increased secretion (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Kudielka, Bellingrath, & Hellhammer, 2006; Kudielka, Hellhammer, & Wust, 2009; Kudielka & Wust, 2010; Miller, et al., 2007). Hypocortisolism was previously interpreted as a favorable outcome and was often described as the protective response of the body to reduce the damage of hypercortisolism (Fries, et al., 2005). However, recent studies have highlighted its detrimental consequences, particularly through its reduced suppressive effect on proinflammatory cytokines, leading to inflammation and fatigue (Bower, Ganz, Aziz, Olmstead, Irwin, & Cole, 2007; Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003; Kumari, et al., 2009; Schrepf, et al., 2013). It is noteworthy that elevated levels of fatigue are particularly important in the context of breast cancer since fatigue has been described has one of the most distressing symptoms reported by cancer survivors (Arndt, Stegmaier, Ziegler, & Brenner; Butt et al., 2007). Thus, hypocortisolism should be considered as much of an undesirable outcome as hypercortisolism.

The measurement of stressful life events

The accumulation of stress experienced by an individual can be measured in a number of ways. Life changes have been suggested to represent stressors since they require adaptation and readjustment on the part of an individual (Dohrenwend, 2006). Scales recording life changes also

offer the benefit of being less affected by response and memory biases than other subjective stress measures and allow the summation of different events to approximate the cumulative effect of stress experienced by an individual (Derogatis, 1982). The value of documenting life changes was originally attributed to the physician Adolph Meyer, who developed a *Life-Chart* to record significant situations and reactions from a patient's life (Meyer & Lief, 1948). Meyer's vision was to better understand the relationship between earlier stressful events and both the onset and the course of a disorder (Meyer & Lief, 1948). Later on, Holmes and Rahe (1967) elaborated a checklist, the Social Readjustment Rating Scale (SRRS), of 43 events that were likely to induce readjustment and changes in a person's typical activities. Examples of the SRRS included a marriage, the death of a spouse, a divorce, trouble with employer, etc. Holmes and Rahe (1967) attributed a fixed weight to each event corresponding to the levels of readjustment associated with the events. They retrieved the weight scores from answers given by a sample of respondents who were asked to approximate to which extent each event would entail life readjustment. Although representing a good starting point in the area of life scales, the SRRS presented certain shortcomings. For example, it did not allow the individualized rating of the impact of each event and did not account for whether the events had a positive or negative impact on the respondent.

In 1978, Sarason, Johnson and Siegel published the Life Experience Survey (LES), a scale that allowed individualised rating of each life event as well as an indication of the valence attributed to each event. The LES included a list of 47 events, some of which were derived from the SRRS and other life stress measures. The LES allows the research participants to indicate which events happened to them during the last year as well as the perceived impact related to each stressor (ranging from extremely negative to extremely positive). The subjective impact of

each event (*Impact Score*) is noted on a seven point Likert scale in which the research participant can indicate the extent to which the event had either a positive impact (1, 2 or 3), a negative impact (-1, -2, -3) or "no impact" (0). Six total scores can be derived from the LES: 1) the total number of stressful life events, 2) the total number of positive events, 3) the total number of negative events, 4) the sum of the *Impact score* given to positive events, 5) the sum of the *Impact score* given to negative events, and 6) the sum of both positive and negative *Impact scores*. The extent to which the LES is affected by social desirability had been measured by Sarason and colleagues (1978) using the Marlow-Crowne Social Desirability Scale (Strahan & Gerbasi, 1972). The correlations between the positive, negative, and total change score of the LES and the total score from the Marlow-Crowne Social Desirability Scale were all non-significant suggesting that the LES is relatively free of the influence of social desirability bias (Sarason et al., 1978). Furthermore, in the past, the LES has been used in research that included a variety of populations such as breast cancer survivors (Kornblith et al., 2001; Kornblith et al., 2003; Lehto, Ojanen, Vakeva, Aromaa, Kellokumpu-Lehtinen, 2008), individuals with graves' disease (Kung, 1995; Topcu, Celik, Tasan, 2012), primary headaches (De Benedittis & Lorenzetti, 1992), individuals recovering from bariatric surgery (Ray, Nickels, Sayeed, & Sax, 2003) and pregnant women (Pluess et al., 2012).

In samples of breast cancer survivors, a greater number of negative stressful life events has been associated with depressive symptoms, poorer quality of life (Lehto, et al., 2008), psychological distress (Kornblith et al., 2001), as well as worse distress, worse posttraumatic stress disorder, and poorer emotional functioning (Kornblith et al., 2003). However, the role of stressful life events on the cortisol secretion patterns of breast cancer survivors has not been yet studied. This relationship has however been investigated in other samples such as mothers of

individuals with autism disorder (Wong, Seltzer, Greenberg, Hong, Almeida, & Coe, 2012) and school aged children (Armbruster, Mueller, Strobel, Lesch, Brocke, & Kirschbaum, 2011). In both studies, higher number of negative stressful life events were associated with lower levels of diurnal cortisol (Wong et al., 2012) as well as lower levels of cortisol following an acute laboratory stressor (Armbruster et al., 2011). Moreover, in Armbruster and colleagues' study, stressful life events rated more severely were associated with smaller cortisol increases following the acute stressor. Thus, stressful life events is an important variable that can contribute to the better understanding of the dysregulation of the cortisol activity and may help to identify which individuals are more at risk of experiencing atypical cortisol secretion patterns.

Objectives of the thesis

The aim of this thesis is to unveil the extent to which breast cancer survivors who have completed all local and/or systemic adjuvant therapy with the exception of hormonal therapy present dysregulated patterns of cortisol secretion. In addition, the role of the cumulative effect of stressful life events on cortisol secretion patterns of research participants was also investigated.

In the first study, the diurnal cortisol rhythms and the stress response of breast cancer survivors and women without a history of cancer was compared. The relationship between medical variables such as time since diagnosis, cancer stage, level of fatigue, and sleep quality were measured in breast cancer survivors. Finally, the subjective levels of stress were also recorded during the stressful procedure to measure if subjective stress levels were similar in breast cancer survivors and women without a history of cancer and paralleled reactive cortisol rhythms.

In the second study, we were interested in exploring the role of stressful life events on the cortisol secretion patterns of breast cancer survivors following an acute stressor. As a first step, we compared the frequency of stressful life events in the past year as well as their subjective impact in breast cancer survivors and women without a history of cancer. As a second step, we evaluated the relationship between the cortisol concentration at peak levels and the amount of stressful life events and their subjective impact reported by our research participants.

Together, these studies were designed to provide a portrait of the HPA activity of breast cancer survivors to appreciate if the experience of cancer can lead to alteration of cortisol rhythmicity long after a cancer diagnosis. The cumulative effect of stress was also documented to determine if it is one of the factors that can interfere with in cortisol rhythmicity of breast cancer survivors beyond that of the cancer diagnosis and treatment.

STUDY 1

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Analysis of the cortisol diurnal rhythmicity and cortisol reactivity in long-term breast cancer survivors

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Abstract

Dysregulation of cortisol rhythmicity has been observed in some breast cancer survivors but whether these alterations persist long after diagnosis has not been well-documented. In the present study, we provide a comprehensive analysis of the diurnal cortisol profiles and cortisol reactivity of breast cancer survivors considered “disease free” compared to women with no history of breast cancer. The results indicate similar diurnal patterns in both groups; however, significant differences in stress reactivity were noted, with breast cancer survivors displaying a relatively flat profile following the acute stress induction. Subjective levels of psychological stress were similar in both groups, suggesting incongruence between perceived stress and the physiological stress response of breast cancer survivors. The patterns suggested a progression towards more typical cortisol reactivity with longer time since diagnosis and may reflect some recovery of HPA axis functioning as time passes.

Introduction

Over the last decades, advances in the fields of screening and treatment have led to the decline in the mortality rate of breast cancer patients. While the increase in survivor rates is encouraging, these results emphasize even more the importance of investigating the psychological and physical well-being of breast cancer survivors later on in their lives. The experience of cancer that encompasses a tumor or tumor cells, difficult treatments, as well as high levels of psychological stress weigh heavily on the biological systems of the body and can lead to their dysregulation (Green McDonald, O'Connell, & Lutgendorf, 2013). One of the systems that has been shown to be particularly altered by the experience of cancer is the HPA axis (Abercrombie et al., 2004; Spiegel, Giese-Davis, Taylor, & Kraemer, 2006; Touitou, et al., 1995; van der Pompe, et al., 1996). However, an important question is whether the HPA axis can recover and go back to its pre-cancer functioning once treatment has terminated and no signs or symptoms of cancer are visible. This is important to address since a compromised HPA axis had been shown to lead to alteration in cardiovascular and immune systems (McEwen & Wingfield, 2003). Furthermore, dysregulation of the HPA axis has been associated with mortality and disease severity in metastatic breast cancer patients (Sephton, et al., 2000) and lung cancer patients (Sephton et al., 2013). In this paper, we examine the long term consequences of a breast cancer diagnosis on HPA axis functioning.

The function of the HPA axis is to maintain homeostasis and prepare organisms to deal with environmental challenges (Fulford & Harbuz, 2005). It has a baseline circadian activity as well as mechanisms designed to respond to an acute stressful situation (Kaltsas & Chrousos, 2007). Via release of cortisol and a cascade of other participants, the HPA axis acts permissively in energy metabolism, stress responsiveness, and information processing (Fulford & Harbuz,

2005). The activity of the HPA axis can be assessed in a variety of ways, most commonly by measuring cortisol output. In healthy individuals, cortisol diurnal secretion follows a typical and predictable slope that peaks 30 to 45 minutes after waking and diminishes throughout the day (Stone, et al., 2001). However, following an acute stressor, cortisol concentration begins to mount and peaks 10 to 30 minutes after stressor cessation and recovery to baseline level is observed 1 to 2 hours afterwards (Foley & Kirschbaum, 2010; Kirschbaum, Pirke, & Hellhammer, 1993).

Circadian patterns of cortisol in breast cancer survivors

Investigation of the circadian rhythm of cancer survivors has received growing consideration over the last decades following its relationship with cancer prognosis and severity (Sephton, et al., 2000). Studies measuring the diurnal rhythms of cortisol in metastatic breast cancer patients have reported an atypical presentation of the cortisol circadian slopes with erratic peaks and troughs as well as flattened profiles (Abercrombie et al., 2004; Mormont & Lévi, 1997; Sephton et al., 2000; Touitou, et al., 1995). Moreover, dysregulation of the circadian rhythm was associated with disease severity (Mormont & Lévi, 1997; Touitou, et al., 1995) and high levels of anxiety (Giese-Davis, et al., 2004). However, no significant alterations were found between the circadian rhythms of recently diagnosed breast cancer survivors (Vedhara, et al., 2006) and early stage breast cancer survivors (Carlson, et al., 2007).

Diurnal rhythms were also investigated in breast cancer survivors who had completed all treatments with the exception of hormonal therapy (Bower, Ganz, Dickerson, et al., 2005; Porter, et al., 2003). In Porter and collaborators' study (2003), breast cancer participants were 3 to 5 years post-diagnosis and most were diagnosed with a cancer stage of 1 or 2. Two sets of saliva samples were collected - a baseline sample one month before a routine mammogram (e.g.

baseline) as well as the day before, the day of a mammogram, and the day after the test. Results indicated that at baseline, breast cancer survivors presented with higher levels of diurnal cortisol than that of women in the control group. However, these levels were lower than the values obtained around the mammogram period. These results suggest that alteration of the diurnal rhythmicity may be present in breast cancer survivors that are considered disease free. Furthermore, these results are consistent with the hypocortisolism hypothesis that suggests lower levels of cortisol in the presence of an acute stressor. Bower and collaborators (2005) investigated the role of fatigue in the cortisol diurnal rhythms of breast cancer survivors considered cancer free. Their research participants were 1 to 5 years post diagnosis. The results indicated that breast cancer survivors with persistent fatigue had flatter diurnal cortisol rhythms compared to their counterparts without fatigue.

Cortisol Reactivity in breast cancer survivors

Cortisol reactivity following an acute stressor was assessed in breast cancer survivors via the use of laboratory protocols design to induce a moderate stress response, such as the Trier Social Stress Test (Bower, Ganz, & Aziz, 2005; Spiegel et al., 2006) and a protocol in which the research participant was asked to prepare a story about a threatening situation (e.g. accused of stealing in a shop; van der Pompe, et al., 1996). The results were mixed. In Spiegel and collaborators' study, metastatic breast cancer survivors were found to have a blunted response to acute stress compared to women without a history of cancer (Spiegel et al., 2006) whereas in van der Pompe et al.'s study, the opposite was found. Finally, in Bower, Ganz, and Aziz's study (2005), the results indicated that the fatigued breast cancer survivors had a significantly blunted stress response compared to that of the non-fatigued group. One of the variables that may account for some of the discrepancy seen in these results relates to how participants perceived

the stressor in these studies. Given the high levels of stress experienced by breast cancer survivors, their appraisal of the level of stress may be different than that of women without a history of cancer (van der Pompe, et al., 1996).

Overall, these results suggest that metastatic breast cancer patients have a more altered pattern of cortisol secretion than that observed in newly diagnosed or early-stage breast cancer patients. However, some alteration of the levels of the diurnal and cortisol reactivity has also been reported in breast cancer survivors who were considered cancer free.

Other factors that may have an impact on cortisol secretion patterns include time since diagnosis, stage of disease, and cancer-related fatigue. While these studies have evaluated breast cancer survivors with less than five years since initial diagnosis, no study has yet investigated the cortisol secretion pattern of long-term breast cancer-free survivors (more than five years since initial diagnosis). Furthermore, no study has combined the assessment of diurnal cortisol and cortisol reactivity in a laboratory setting in disease-free breast cancer survivors and women without a history of cancer.

Using a cross-sectional design, the aim of the present study was to provide a comprehensive analysis of the diurnal cortisol secretion profiles and their patterns following an acute stressor in breast cancer survivors with no evidence of disease otherwise and to compare these to women with no history of breast cancer. Factors associated with breast cancer were also assessed in order to evaluate their relationship with cortisol patterns; these included time since diagnosis, cancer stage, fatigue, sleep quality, and subjective levels of psychological stress. The unique contribution of this study is the inclusion of perceived stress and both diurnal and reactive patterns of cortisol using a standardized laboratory protocol in the same individuals.

Methods

Participants

Breast cancer survivors and women without a history of breast cancer were recruited for this study. To be included in the breast cancer survivor group, women needed to satisfy the four following inclusion criteria: 1) diagnosis of breast cancer, 2) completion of all local and/or systemic adjuvant therapy at least six months earlier, with the exception of hormonal treatment, 3) considered cancer free, and 4) fluent in English. Exclusion criteria were the following: 1) no previous history of other cancers, with the exception of non-invasive skin cancer and cervical cancer and 2) no other major disabling medical or psychiatric conditions that could interfere with quality of life. Inclusion criteria for the control group were the following: 1) women aged between 29 and 80 years old, and 2) fluent in English. Exclusion criteria for the control group were the following: 1) no previous history of cancers, with the exception of non-invasive skin cancer and cervical cancer and 2) no other major disabling medical or psychiatric condition that could interfere with quality of life.

Instruments

Trier Social Stress Test (TSST)

The TSST is a widely used tool designed to induce moderate stress in a laboratory setting (Kirschbaum, 1993). It is a task that combines high levels of social-evaluative threat and uncontrollability (Dickerson & Kemeny, 2004). The TSST consists of a mock interview in which the research participant is asked to deliver a free-speech as well as to perform an arithmetic task in front of a panel of judges that provides no feed-back (Kirschbaum, et al., 1993). The TSST has been found to be very effective in inducing a cortisol response, with roughly a two to threefold

rise in salivary cortisol levels in a majority of tested participants (Kudielka, Hellhammer, & Kirschbaum, 2007).

Salivary Cortisol

Saliva samples were assayed in duplicate for cortisol using commercially available highly-sensitive enzyme linked immunosorbent assay kits and the protocol designed by Salimetrics, State College, PA.

Visual Analog Scale (VAS)

The VAS is a 100mm bipolar line that measures a characteristic across a continuum. Participants mark a spot on the line that indicates their subjective feeling (Aitken, 1969). In the present study, subjective appraisal of stress was measured with the statement "I feel stressed". The scale was anchored from 0 = "not at all" to 100 = "very much". Scores were determined by measuring the distance from the left end to the appraisal mark.

Bidimensional Fatigue Scale (BFS)

The BFS is a questionnaire developed by Chalder and colleagues (1993). It has 11 items with seven items assessing physical fatigue and four items assessing mental fatigue. Each item is answered on a 4-point scale and the total scores can range between 0 and 33 with higher scores indicating greater fatigue. The BFS also has a cut-off score of 11 meaning that scores of 11 and greater indicate significant fatigue (Alexander, et al., 2009).

Sleep Quality

The participants were asked to report the number of hours they slept the night before Day 1, the night before Day 2, and the night before their laboratory visit. The average number of hours of sleep was calculated for each participant and was used as an indicator of their sleep quality.

Socio-demographic questionnaire

The socio-demographic questionnaire entailed questions regarding general history, health history, and habits known to influence cortisol concentrations. In addition, the breast cancer survivors were also questioned regarding their breast cancer history.

Procedure

This study comprised two parts. For the first part, each participant was asked to provide five saliva samples at home at specific times (waking, 30 minutes after waking, noon, 16h00 and 21h00) on each of two consecutive days. The purpose was to assess the cortisol diurnal pattern. For the second part, each participant was asked to participate in a laboratory protocol (the TSST) designed to elicit a moderate stress response as indexed by a change in baseline cortisol levels.

Participants were recruited via multiple sources to target both breast cancer survivors and women with no history of cancer in a similar age range. Ads were placed on radio, in newspaper and magazines. Those who had indicated an interest in the study were contacted via phone. During the first telephone conversation, a summary of the study was provided and eligibility of potential participants was verified. Eligible participants were scheduled at their convenience for two laboratory visits at the University of Ottawa. The first visit served to obtain informed consent and to provide specific instructions on how and when to collect at-home saliva samples. Participants were provided with a container of labelled salivettes to use at each time point. To collect the samples, participants were asked to rinse their mouths with water and to wait at least 10 minutes before collection to avoid sample dilution. They were instructed to place the swab directly under their tongues and to wait for three minutes, after which the swab was returned to the salivette and stored in the refrigerator. Participants were asked to avoid alcohol for 24 hours before sample collection, to refrain from eating a major meal within 60 minutes of sample

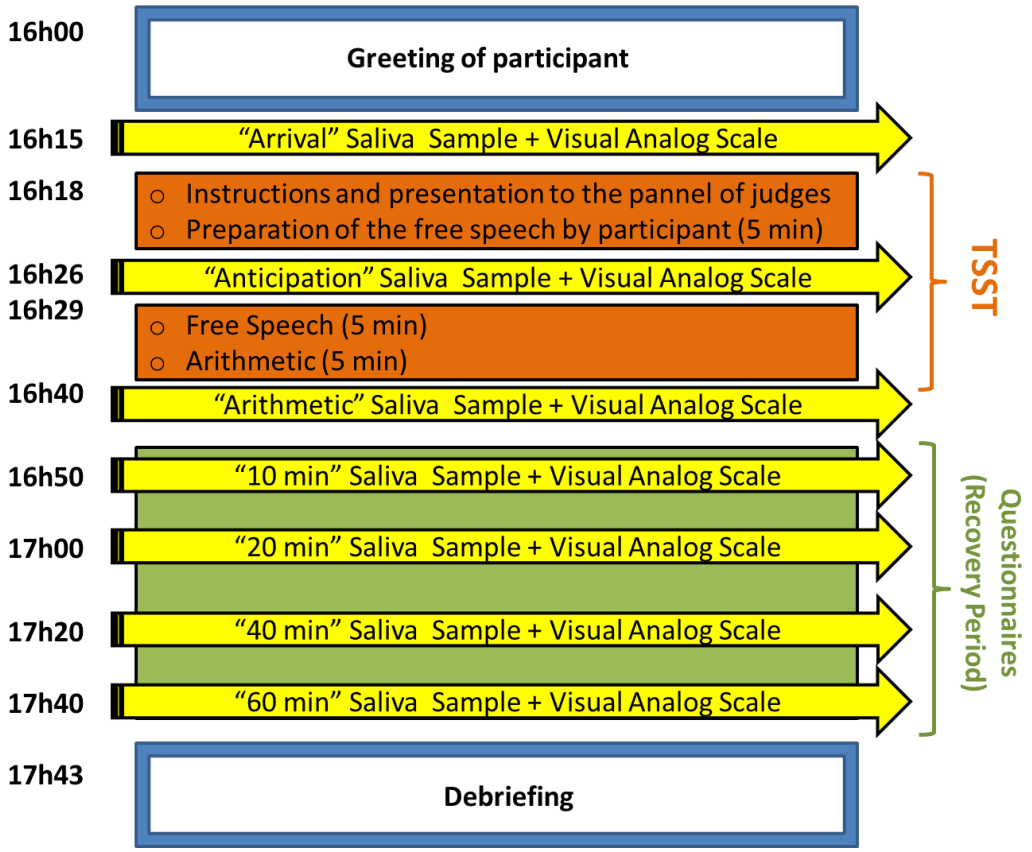
collection, to avoid dairy products for 20 minutes before sample collection, and to avoid foods with high sugar or acidity (citrus), or caffeine content, immediately before sample collection. They were also asked to avoid exercise/working out/training 1 hour before sample collection and to avoid brushing their teeth immediately before sample collection (guidelines based on Salimetrics protocol; Salimetrics, 2008). Participants were provided with a booklet in which they were asked to indicate the time at which they collected the sample as well as any associated mistakes. At the end of the first visit, the second laboratory visit was scheduled, roughly within seven days after completion of the in-home saliva collection.

Second visit - Laboratory Stress Protocol

Figure 1 presents an overview of the second laboratory visit. The visit was scheduled at the end of the day, beginning between 15h00 and 17h00, and lasted approximately 2 hours; this period was chosen to coincide with the time at which cortisol concentrations are at their lowest values and the function relating time to cortisol concentration is relatively flat. On the day of the laboratory visit, the participant was escorted to the test room and invited to share the saliva collection experience and report any problems. The participant was then asked to drink a glass of water and relax for ten minutes. The consumption of water was added to the protocol to increase the odds of having sufficient saliva for sampling during the TSST. The participant was then asked to provide a saliva sample (sample labelled "arrival") and to rate her subjective stress level on the VAS. Once completed, the participant was led to a second test room in order to introduce the mock panel of judges and give instructions on the free speech. The participant was then given five minutes to prepare this task, after which she was asked to provide another saliva sample (sample labelled "anticipation") and prompted to rate her stress level on the VAS. This was followed by delivery of the free speech and participation in an arithmetic task in front of the

panel of judges. After completion of these two tasks, a third saliva sample (sample labelled "arithmetic") was requested and the participant prompted to rate her stress level on the VAS. Afterwards, the participant was asked to complete a series of questionnaires, such as the BFS and the socio-demographic questionnaire and to relax for a period of 1 hour. During that time, the participant was requested to provide four additional saliva samples along with a VAS rating at 10, 20, 40, and 60 minutes after the end of the TSST. Once the last sample was collected, there was a debriefing period during which the TSST was explained and the participant invited to share her experience. Upon the departure of the participant, all saliva samples were transferred in Eppendorf tubes and stored in a freezer at -80 °C for further analysis.

Figure 1.



Statistical analyses

T-tests were used to assess group differences in age and fatigue level, and as post-hoc tests following standard mixed design ANOVA analyses on diurnal and reactive cortisol data, and the VAS scores related to subjective stress. The post-hoc analyses were based on peak TSST and area under the curve values and trend analyses (cortisol reactivity data) and area under the curve in the case of the diurnal cortisol data. Correlation analyses were used to determine relationships between specific medical variables. Finally chi-square tests evaluated the frequency of individuals who reported their status as either pre- or post-menopausal.

Results

Participants

Demographic characteristics

Twenty-two breast cancer survivors and 25 women without a history of breast cancer completed both parts of the study. One breast cancer survivor provided saliva samples for the diurnal analysis but was unable to take part in the TSST and therefore did not complete any of the questionnaires. The range and distribution of ages was similar in both groups with no significant difference in mean age (t = -0.700, p = 0.488). The proportion of women with a post-menopausal status was also similar in both groups with no significant difference (x^2=2.101, p=0.147) as was the average number of hours of sleep; women in the control group had an average of 7.6 hours (SD = 1.09), while breast cancer survivors had an average of 7.2 hours (SD = 0.83; t = 1.391, p = 0.172). The majority of participants were Caucasian, in a relationship, had completed an undergraduate degree, and in a white collar profession or retired. A complete list of the demographic characteristics of participants is shown in Table 1.

Medical characteristics

The participants' medical characteristics are presented in Table 2. Two women in the breast cancer group had experienced a recurrence in breast cancer prior to the study; one women reported one recurrence and the other, two recurrences. The information in Table 2 is based on the most recent breast cancer diagnosis of the participants. The treatment history was quite diverse, including chemotherapy, radiation, and hormone therapy, either alone or in some combination. There were no serious or untreated co-morbid medical conditions. Most commonly reported was hypertension, followed by one or two cases of diabetes per group, and several individuals with osteoarthritis in the breast cancer participants. None of these, if treated, have an impact on reactive cortisol, to our knowledge. Finally, none of the research participants reported being treated with cortisone medication.

Table 1. **Demographic characteristics of the breast cancer and control groups**

Demographic characteristics	Breast cancer survivors N = 22	Control group N = 25
Mean age ± SD (yr)	59 ± 10	57 ± 11
Age range (yr)	39 - 81	29 - 71
	%	**%**
Post-menopausal status	83	64
Ethnicity		
Caucasian	91	88
Black	---	4
Asian	---	8
First Nations	9	---
Marital status		
Single	9	8
Married/civil union/common law	68	56
Separated/divorced	18	28
Widowed	5	8
Highest level of education		
High school	27	32
College	18	16
Bachelor's degree	50	28
Master's degree	5	20
Doctoral degree	---	4
Employment status		
Blue collar	---	8
White collar	50	24
Self-employed	4	16
Homemaker	14	---
Medical leave of absence	---	4
Retired	32	48
Family Income		
Under $40,000	15	22
$40,000-99,999	60	52
$100,000-199,999	25	26

Table 2. **Medical characteristics of the breast cancer group**

Medical characteristics	Breast cancer survivors N = 22
Mean age at diagnosis ± SD (yr)	54 ± 9
Age range (yr)	36 – 76
Mean time since diagnosis ± SD (yr)	4.6 ± 3
Time since diagnosis range (yr)	1.2 – 11.8
	%
Breast cancer stage	
0	18
1	45
2	23
3	14
Type of surgery	
Unilateral mastectomy	27
Bilateral mastectomy	32
Lumpectomy	41
Chemotherapy treatment	
Yes	46
No	54
Hormone therapy	
Yes	64
No	36
Radiation therapy	
Yes	64
No	36

Diurnal saliva sample collection

The times at which saliva samples were collected at home in both groups are presented in Table 3. All participants presented good compliance to collection instruction and no significant group differences were found at any time-point.

Table 3. **Average ± SD collection time of diurnal saliva samples in each group**

	Breast cancer survivors		Control group	
	Day 1	Day 2	Day 1	Day 2
Waking	6:53 ± 1.1 h	6:49 ± 1.0 h	7:10 ± 1.2 h	7:17 ± 1.4 h
30 min after waking	7:25 ± 1.2 h	7:22 ± 1.0 h	7:43 ± 1.2 h	7:44 ± 1.4 h
Noon	12:07 ± 0.2 h	12:08 ± 0.2 h	12:06 ± 0.2 h	12:16 ± 0.6 h
Four	15:41 ± 2.6 h	15:49 ± 2.8 h	16:09 ± 0.6 h	16:14 ± 0.5 h
Nine	20:49 ± 2.6 h	21:09 ± 0.3 h	21:05 ± 0.5 h	21:14 ± 0.5 h

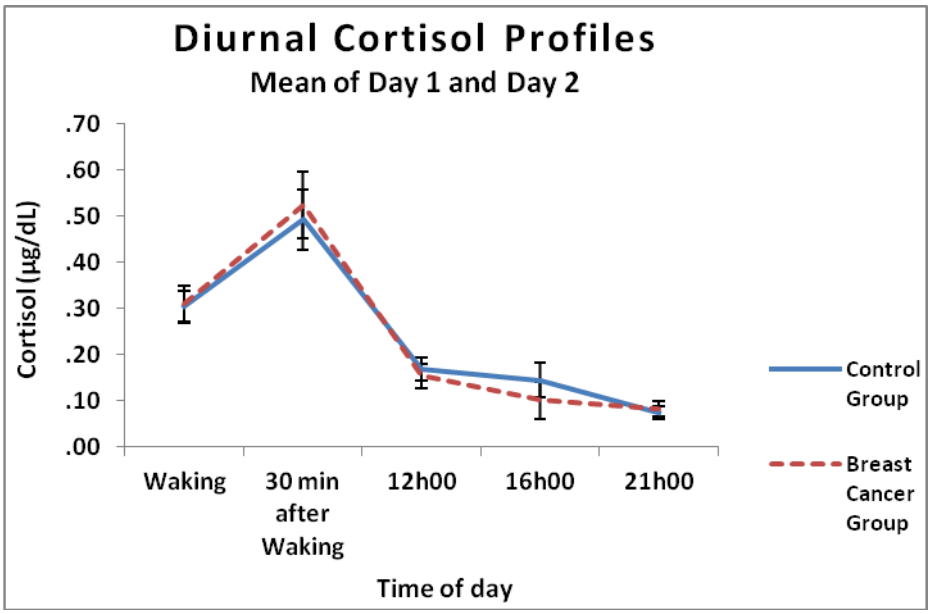
No significant differences were found between collection time of breast cancer survivors and women in the control group at each time point on each day (p value ranged from 0.199 to 0.731)

Diurnal cortisol

A mixed-design ANOVA was performed to assess group, time, and group X time differences in mean cortisol concentrations over two consecutive days at five time points: waking, 30 minutes after waking, noon, 16h00, and 21h00. The between-subjects factor consisted of two levels (breast cancer survivor and control group) while time, the repeated factor, had five levels. The pattern observed in Figure 2 indicates that both breast cancer survivor and control groups followed a similar and typical pattern of diurnal cortisol secretion with peak concentrations at 30 minutes after awakening and gradual reduction of cortisol throughout the day. The analysis gave rise to a significant main effect of time ($F = 51.427$, $p < .001$) but no group ($F = .005$, $p = 0.945$) or interaction difference ($F = .320$, $p = 0.721$). The within-case area

under the curve was calculated using trapezoidal integration. A t-test indicated no significant difference between groups in this measure (t = 0.116, p= 0.908).

Figure 2.



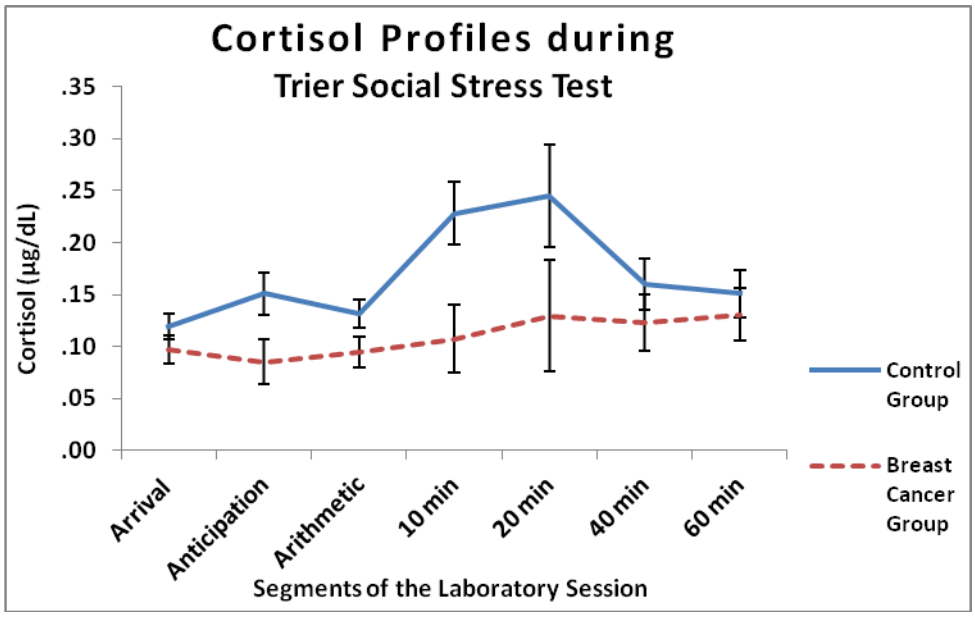
Cortisol reactivity

The patterns of cortisol reactivity were assessed via the TSST at seven time points (arrival, after the anticipation segment of the TSST, after the arithmetic segment of the TSST and 10, 20, 40, and 60 minutes after the end of the TSST. The results of the mixed-design ANOVA indicated a significant main effect of time (F = 3.282, p = 0.031) and group (F = 4.884, p = 0.033), but no significant interaction between the two factors (F = 1.703, p = 0.179). Removing the two breast cancer participants who experienced recurrences altered the probability of the group effect to a p value of 0.052.

A test of within-subjects contrasts indicated a significant quadratic effect in control participants (F = 4.851; p=0.040) but no such pattern in breast cancer survivors (F = .168;

p=0.687). We also calculated the within-case area under the curve using trapezoidal integration. A t-test indicated a significant group difference in the area under the curve (t = 2.251, p= 0.03). As can be seen in Figure 3, women in the control group presented with a typical reactivity slope with peak concentrations at 10 to 20 minutes following the stressor and recovery to baseline levels. In comparison, women in the breast cancer survivor group showed a blunted cortisol response following the stressor with a relatively flat slope.

Figure 3.

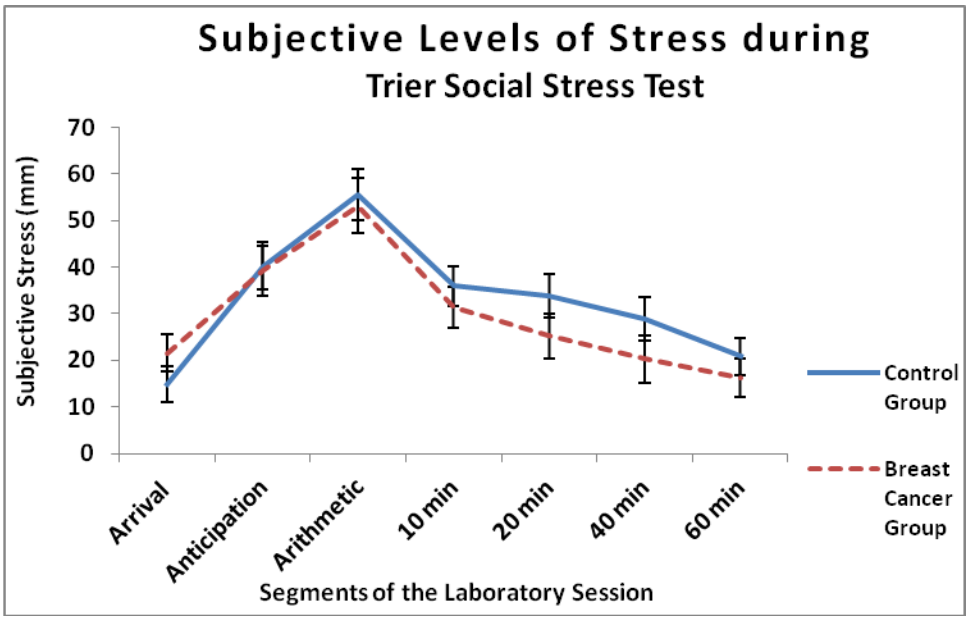


Subjective levels of stress during Trier Social Stress Test

Subjective levels of stress were assessed during the TSST using the VAS (Figure 4). Participants were asked to rate their level of stress exactly at the same time as saliva samples were collected. The results of the mixed-design ANOVA on these subjective responses indicated a significant main effect of time (F = 19.419, p < .001) but no group (F = .703, p = 0.406) or

interaction (F = .810, p = 0.493) effects. A follow-up One-way ANOVA gave rise to no significant group difference in the area under the curve of all groups (F = 0.472, p = 0.627).

Figure 4.

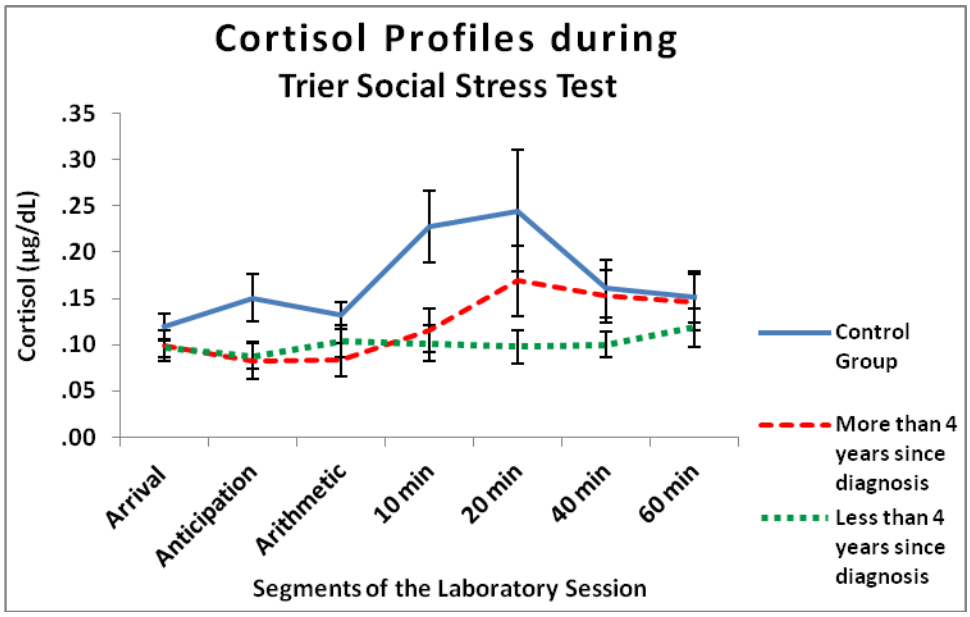


Cortisol reactivity profiles and medical variables

Additional analyses were performed to evaluate the relationship between breast cancer characteristics and the cortisol profile observed during the TSST. Participants in the survivors group were organised into two groups based on the number of years since their breast cancer diagnosis - “less than four years” and “more than four years”. The number of years chosen was based on the statistical median of our sample (four years). A mixed-design ANOVA was performed to assess group, time, and group X time differences in cortisol concentrations during the TSST. The between subjects factor consisted of three levels (“breast cancer survivor less than four years since diagnosis”, “breast cancer survivors more than four years since diagnosis” and “women in the control group”) while time, the repeated factor, had seven levels. The results

indicated no significant main effect of time (F = 2.029, p = 0.123), group (F = 2.523, p = 0.094) or interaction (F = 1.061, p = 0.389) effects. A planned comparison of the peak value which occurred at 20 minutes following the TSST indicated a group difference that approached significance between women in the "more than four years since diagnosis" and "less than four years since diagnosis" groups (t = -1.809, p = 0.089). The difference in the area under the curve between the breast cancer survivors less than four years since diagnosis and four years or more since diagnosis was not significant (t = .945, p= 0.359). A test of within-subjects contrasts indicated a quadratic effect in breast cancer survivors who were diagnosed more than 4 years ago (F = 5.154; p=0.057) while no such pattern was seen in breast cancer survivors diagnosed less than 4 years ago (F = 0.581; p=0.465). These data are displayed in Figure 5.

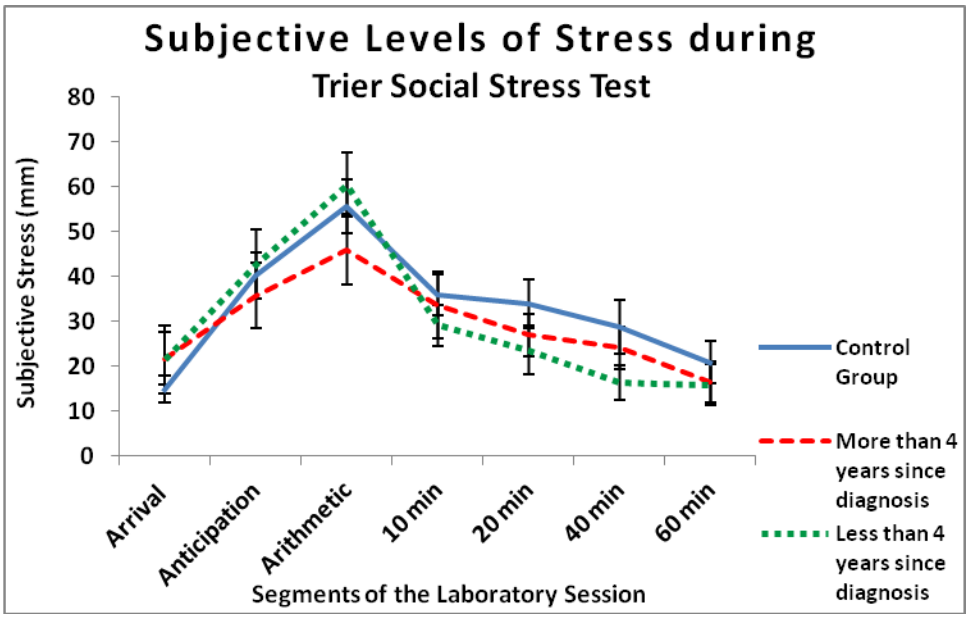
Figure 5.



Subjective levels of stress were also evaluated to verify if the induced stress level by the TSST was perceived in a similar fashion in all groups on the VAS. The results of the mixed-

design ANOVA on these subjective responses indicated a significant main effect of time (F = 16.868, $p < .001$) but no group (F = .351, p = 0.706) or interaction (F = .806, p = 0.573) effects. A t-test gave rise to no significant group difference in the area under the curve (t=0.159, p= 0.875). The pattern seen in Figure 6 suggests that the women in all three groups experienced the expected psychological reaction to stress with higher levels perceived during the TSST and a gradual reduction following the end of the stressor.

Figure 6.



To examine the role of cancer stage and reactive cortisol patterns as indexed by area under the curve, we subdivided the breast cancer participants into low (0 and 1) and high (2 and 3) cancer stages and determined if there were group differences on this measure; none was found (t = 0.391, p = 0.701).

We also evaluated the relationship between fatigue and the diurnal cortisol secretion pattern in both groups. First, using the cut-off score of "11 or more" on the BDF, the majority of

the participants in both groups had a level of fatigue that would be considered significantly high: 88% of women without a history of cancer had a total fatigue score of 11 or more (mean = 12.63, SD= 3.32, range = 8 - 23) and 84% of breast cancer survivors had a total fatigue score of 11 or more (mean = 13.11; SD=5.43; range= 1 - 24). A t-test indicated no difference between groups in the total level of fatigue (t = -0.310, p = 0.759) as well as in the level of physical fatigue (t = 0.174, p = 0.863) and mental fatigue (t = -0.446, p = 0.659). Follow-up correlational analysis revealed no relationship between total levels of fatigue and cortisol during the TSST in both breast cancer survivors (p value ranged from 0.278 to 0.794) and women in the control group(p values ranged from 0.123 to 0.727).

Finally, Pearson r correlations were performed to evaluate the relationship between time since diagnosis, the breast cancer stage at diagnosis, and the total level of fatigue in breast cancer survivors. No significant correlation was found in any of the variables (p values ranged from 0.297 to 0.462).

Discussion

The goal of this study was to evaluate if long-term breast cancer survivors are at risk of presenting with a dysregulated HPA axis functioning compared to women without a history of cancer in the similar age range. We assessed the functioning of the HPA axis by measuring the cortisol secretion patterns during the day as well as its reactivity profile following an acute stressor. Our results indicated that while the diurnal patterns of breast cancer survivors appeared typical and very similar to women in the control groups, their cortisol reactivity patterns were significantly altered, that is, characterized by a blunted response. Subjective levels of psychological stress monitored during the Trier Social Stress Test protocol indicated that both breast cancer survivors and women in the control group reported feeling stressed during the

stress protocol, especially during the free speech and arithmetic tasks. Women in both groups also reported a diminution of reported levels of psychological stress during the recovery period. These results suggest that breast cancer survivors appear to appraise psychological stress in a similar fashion to that of women without a history of cancer but have a diminished physiological stress response to that event. The hyporeactivity of the HPA axis has been observed in some breast cancer survivors (Bower, Ganz, & Aziz, 2005; Spiegel et al., 2006) as well as in other individuals experiencing chronic stress (Miller, et al., 2007). Among the plausible mechanisms that have been suggested, chronic inflammation related to the experience of cancer has been argued as one of the factors that could lead to dysregulation of the HPA axis in cancer survivors (Bower, Ganz, Dickerson, et al., 2005; van der Pompe, et al., 1996). In their study, Bower, Ganz, Dickerson and collaborators (2005) noted that fatigued breast cancer survivors had a flatter cortisol reactivity slope than did non-fatigued breast cancer survivors. These results are important since the majority of our participants were significantly fatigued based on the cutoff score of the Bidimensional Fatigue Scale (Alexander et al., 2009); however this level applied to both survivors and control subjects. Another mechanism that has been proposed refers to the idea that the experience of cancer can be seen as a situation of chronic stress. Numerous studies have suggested that breast cancer survivors experience high levels of stress at the beginning of their diagnosis and that this distress may persist several years after diagnosis under the form of the fear of cancer recurrence (Deimling, et al., 2006; Lim, et al., 2011; Montgomery & McCrone, 2010; Vickberg, 2003). Hans Seyle was one of the first to suggest that the functioning of the stress system was optimal during punctual rather than chronic stress situations. Thus, chronic activation of the stress system was suggested to lead to its dysregulation. The concept of allostasis and allostatic load, popularised by McEwen and colleagues (McEwen, 1998; McEwen

& Wingfield, 2003) can prove to be a good framework in the unravelling of the relationship between stress and cortisol patterns in chronically stressed individuals. It suggests that the HPA axis plays a crucial role in the maintenance of homeostasis under stressful situations. Thus, when there is a prolonged accumulation of stressors, chronic activation of the HPA axis may lead to its "wear and tear" and potentially be associated with a reduced physiological response.

We also evaluated the role of medical variables such as time since diagnosis and cancer stage in altered cortisol reactivity patterns in breast cancer survivors. Albeit not significant, the overall profile suggests that cortisol reactivity increases or normalizes with the passage of time following the breast cancer diagnosis. No such pattern was observed when this analysis was applied to participants divided into low (0 and 1) and higher cancer stages (2 and 3). The relationship between medical characteristics and positive outcomes needs to be thoroughly established and should be examined more closely in terms of other health issues associated with chronic HPA axis dysregulation.

Limitations

It is recognized that because convenience sampling was conducted, the participants in this study may not adequately represent all breast cancer survivors. They were recruited locally, generally from urban areas, and well-educated. Finally, it should be noted that our breast cancer participants presented with a variety of cancer treatments - chemotherapy, radiation, hormonal therapy or some combination of two or three of these treatments. The sample sizes of the sub-groups were too small to consider further analysis. Future studies should investigate the influence of treatment on HPA axis regulation in breast cancer survivors. Ideally, physical examinations and clinical assessments would be included such as endocrine tests to rule out factors that may affect HPA axis functioning.

Conclusion

In summary, our results indicated normal diurnal cortisol patterns and stress experiences in breast cancer survivors but significant differences in acute stress reactivity. Their cortisol patterns following stress induction were characterized by relatively flat profiles compared to healthy individuals and these appeared to be somewhat associated with time since diagnosis. We are following up in other studies to evaluate other stress-related and immune biomarkers in order to understand the relationship between HPA axis functioning and long-term health in this population.

STUDY 2

The Role of Stressful Life Events on the Cortisol Secretion Patterns of Breast Cancer Survivors

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Abstract

Atypical patterns of cortisol secretion following an acute stressor have been commonly reported among breast cancer survivors. Stressful life events have been associated with lower levels of cortisol in other populations and have been found prevalent in samples of breast cancer survivors. The purpose of this study was to explore the role of stressful life events on the cortisol secretion patterns of breast cancer survivors following an acute stressor. We recorded the frequency of stressful life events in the past year as well as their subjective impact using the Life Experience Survey in 19 breast cancer survivors and 17 women without a history of cancer. The Trier Social Stress (TSST) was used to elicit a moderate stress response and saliva samples were collected before during and after the TSST to provide cortisol concentrations. Results suggest no group differences between the total number of stressful life events and their subjective impact. However, the total number of stressful life events as well as their subjective impact correlated negatively with the peak cortisol concentration in breast cancer survivors. The results suggest that the cumulative effect of stressful life events may impact the endocrine stress system of breast cancer survivors more so than that of women with no history of cancer. These results are discussed in the context of the long term management of health in breast cancer survivors.

Introduction

Breast cancer is one of the most common forms of cancer diagnosed in women in North America (American Cancer society, 2014; Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014) with roughly 12% of women expected to develop breast cancer in their life time (American Cancer society, 2013; Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). On a more positive note, advances in screening and treatment methods have led to a decline in the mortality rate of breast cancer patients; current figures indicate a survival rate of more than five years following the initial cancer diagnosis in approximately 89% of women (American Cancer society, 2013; Canadian Cancer Society's Advisory Committee on Cancer Statistics, 2014). These results emphasize the importance of studies related to the psychological and physical well-being of breast cancer survivors later on in their lives. One aspect of the breast cancer experience that may influence both the psychological and physical health of breast cancer survivors is the prevalence of higher levels of psychological stress in that population (Deimling et al., 2006; Lim, Devi, and Ang, 2011; Mehnert and Koch, 2007; Montgomery & McCrone, 2010).

A large body of literature suggests that women diagnosed with breast cancer usually present higher levels of psychological stress and anxiety across the disease trajectory. A recent review by Montgomery and McCrone (2010) indicated that up to half of women experience high levels of psychological distress during the diagnostic period. In another study by Mehnert and Koch (2007), significant levels of anxiety were noted in 39.6% of breast cancer survivors following their first breast cancer surgery, while 32.7% of breast cancer survivors still reported high levels of anxiety six months post-surgery. Likewise, Mehnert and Koch (2008) found that

38% of breast cancer survivors, being on average three years post-diagnosis, had moderate to high levels of anxiety and that 12% had cancer-related post-traumatic stress disorder.

In long-term breast cancer survivors (5-34 years post-diagnosis), similar results have been found suggesting that approximately one third of breast cancer survivors continue to report worries about a cancer recurrence (Deimling et al., 2006). Altogether, these studies suggest that a majority of breast cancer survivors are confronted with high levels of psychological stress at the beginning of their disease and that this distress may persist several years after the initial cancer diagnosis. Thus, the experience of breast cancer represents a situation of chronic stress. While a punctual period of stress can be beneficial to the organism, chronic stress may adversely interfere with the normative activity of the stress system.

One of the main components of the stress system is the hypothalamo-pituitary-adrenal (HPA) axis. When a stressor is perceived by an organism, a cascade of predictable steps is initiated, starting with the activation of neurons located in the paraventricular nucleus of the hypothalamus that are responsible for the release of adrenocorticotrophic hormones in the pituitary gland; this in turn activates the secretion of glucocorticoids, a class of steroid hormones produced by the adrenal glands, the most important of which is cortisol (Herman, 2011; Thiel & Dretsch, 2011). Cortisol plays a primary role in controlling the stress response, among its many other regulatory functions that are essential for life. Following the activation of the stress response, cortisol helps to regulate the functioning of the HPA axis by creating a negative feed-back loop that resets the system to its initial state (Herman, 2011; Thiel & Dretsch, 2011).

The cortisol secretion patterns following an acute stressor have been well-studied (Foley & Kirschbaum, 2010; Kudielka & Wust, 2010; Kudielka, Hellhammer, Wust, 2009). In most individuals in good psychological and physical health, a two to threefold increase in salivary

cortisol is noted around 10 to 20 minutes following the cessation of a laboratory-induced stressor with a return to baseline levels usually within two hours (Kirchbaum, Pirke, & Hellhammer, 1993; Kudielka & Wust, 2010).

The literature on the effect of chronic stress on HPA axis activity has evolved over the years. Selye (1978) was one of the first researchers to highlight the idea that while the stress response is overall adaptive, it can also threaten the organism via the hyperactivation of the adrenal gland. Following his pioneer work, it was generally assumed that chronic stress would be associated with hyperactivation of the HPA axis and consequently higher levels of cortisol. There are numerous studies on the phenomenon of hypercortisolism in chronically stressed individuals (Gold, et al., 2003; Gonzalez-Cabrera, et al., 2014; Heim, et al., 2000; Laufer, et al., 2012; van der Hal-Van Raalte, et al., 2008). However, in the last decades, the reverse has also been observed, that is, hypocortisolism, or low cortisol levels in individuals who have undergone long-term exposure to psychological stress (Bergen et al., 2012; Juster, et al., 2011; Gordis, et al., 2008; Matthews, et al., 2001) as well as early life trauma (Carpenter, et al., 2011; Engert, Efanov, Dedovic, Duchesne, Dagher, & Pruessner, 2010; Gunnar, et al., 2009; Macmillan, et al., 2009; Trickett, et al., 2014). One explanation for these results that has been proposed is that following a prolonged period of excessive cortisol secretion, hypocortisolism acts as a protective response to reduce the damage of chronic elevations of cortisol (Fries, Hesse, Helhammer, & Hellhammer, 2005; Kudielka, Bellingrath, & Hellhammer, 2006; Kudielka, Hellhammer, & Wust, 2009; Kudielka & Wust, 2010; Miller, Chen, & Zhou, 2007). While once understood as a favorable outcome, recent studies have highlighted the detrimental consequences of hypocortisolism, particularly through its reduced suppressive effect on proinflammatory cytokines, leading to inflammation and high levels of fatigue (Bower, Ganz, Aziz, Olmstead,

Irwin, & Cole, 2007; Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003; Kumari, et al., 2009; Schrepf, et al., 2013). Elevated levels of fatigue are particularly important in the context of breast cancer since fatigue has been described as one of the most distressing symptoms reported by cancer survivors (Arndt, et al., 2006; Butt et al., 2007). Thus, hypocortisolism may be as much an undesirable consequence as hypercortisolism.

In samples of breast cancer survivors, lower levels of cortisol following an acute stressor have been reported in metastatic breast cancer survivors (Spiegel, Giese-Davis, Taylor, & Kraemer, 2006; van der Pompe, Antoni, Heijnen, 1996), breast cancer survivors scheduled for a routine mammogram (Porter, Mishel, Neelon, Belyea, Pisano, & Soo, 2003), fatigued breast cancer survivors (Bower, Ganz, & Aziz, 2005) and breast cancer survivors treated with the GnRH agonist Lupron, a type of hormonal therapy, following a painful stressor (Andreano, Waisman, Donley, & Cahill, 2012).

The association between psychological stress and cortisol in breast cancer survivors has been disputed in the past. While in some studies, no clear associations have been found between psychological variables and the cortisol diurnal patterns of breast cancer survivors (Carlson, Campbell, Garland, & Grossman, 2007; Vedhara, Tuinstra, Miles, Sanderman, & Ranchor, 2006), high levels of anxiety have been linked to flatter levels of cortisol following an acute stressor (Giese-Davis, Sephton, Abercrombie, Duran, & Spiegel, 2004). Furthermore, in Porter and colleagues' research (2003), breast cancer survivors displayed lower levels of cortisol on the days surrounding a routine mammogram, suggesting an association between higher levels of psychological stress and lower levels of cortisol in that population.

The quantification of chronic psychological stress experienced by an individual can be assessed by measuring the occurrence of specific stressors and their subjective impacts or by

assessing one's level of stress through different time periods such as the last 24 hours (Brantley, Waggoner, Jones, & Rappaport, 1987), the last month (Cohen, Kamarck, & Mermelstein, 1983) and the last year (Sarason, Johnson, & Siegel, 1979). Recording the accumulation of specific stressors and their subjective impacts through a full year offers the benefit of capturing the levels of stress experienced by an individual during a longer period of time and offers a better appreciation of a situation of chronic stress.

Sarason, Johnson and Siegel (1978) created the Life Experience Survey (LES), a questionnaire that includes a list of common stressors from which research participants can select those that occurred during the last year as well as the perceived impact related to each stressor (ranging from extremely negative to extremely positive). Examples of stressors include a change of residence, marriage, separation, retirement, the departure of a child, etc. In the past, the LES has been used to assess stressors included in samples of breast cancer survivors (Kornblith et al., 2001; Kornblith et al., 2003; Lehto et al., 2008), individuals with graves' disease (Kung, 1995; Topcu et al., 2012), primary headaches (De Benedittis & Lorenzetti, 1992), recovery from bariatric surgery (Ray, Nickels, Sayeed, & Sax, 2003) and in pregnant women (Pluess et al., 2012). In breast cancer survivors, a greater number of negative stressful life events has been associated with depressive symptoms, poorer quality of life (Lehto, et al., 2008), psychological distress (Kornblith et al., 2001), as well as worse distress, worse posttraumatic stress disorder, and poorer emotional functioning (Kornblith et al., 2003).

The role of stressful life events on the impact of cortisol secretion patterns in breast cancer survivors has not been studied, although this relationship has been investigated in other populations such as mothers of individuals with autism disorder (Wong, Seltzer, Greenberg, Hong, Almeida, & Coe, 2012) and school aged children (Armbruster, Mueller, Strobel, Lesch,

Brocke, & Kirschbaum, 2011). In both of these studies, higher number of negative stressful life events were associated with lower levels of diurnal cortisol (Wong et al., 2012) as well as lower levels of reactive cortisol following an acute laboratory stressor (Armbruster et al., 2011). Moreover, in Armbruster and colleagues' study, stressful life events rated more severely were associated with a smaller increase of cortisol following the acute stressor.

In our previous study, we assessed the cortisol secretion patterns of long-term breast cancer survivors (Couture-Lalande, Lebel, & Bielajew, 2014). Following a laboratory protocol designed to elicit a moderate stress response, the Trier Social Stress Test (TSST), lower levels of cortisol secretion were found in breast cancer survivors while women without a history of cancer presented with the typical pattern of increased levels of cortisol. In this paper, we are interested in exploring the role of stressful life events and their perceived impact on the blunted cortisol stress response noted in our group of breast cancer survivors.

Methods

Participants

Research participants comprised a group of breast cancer survivors and a group of women without a history of breast cancer. The inclusion criteria for the breast cancer survivors were as follows: 1) a diagnosis of breast cancer, 2) completion of all local and/or systemic adjuvant therapy at least six months earlier, with the exception of hormonal treatment, 3) considered cancer free (no signs and symptoms of cancer), and 4) fluent in English. Participants were excluded if they had 1) a previous history of other cancers, with the exception of non-invasive skin cancer or cervical cancer, and 2) were diagnosed with other major disabling medical or psychiatric conditions that could interfere with quality of life. We recruited women

for the comparison group who were between 29 and 80 years old, fluent in English with no previous history of cancer, and otherwise the same exclusion criteria as above.

Instruments

Trier Social Stress Test (TSST)

The TSST is a standardised laboratory protocol designed to induce a moderate stress response (Kirschbaum, Hellhammer, & Dirk, 1993). The participant is asked to prepare a speech that is then delivered in front of a panel of judges who have been especially trained to deliver no verbal or non-verbal feed-back; this is followed by a challenging arithmetic task in front of the same panel. The TSST has been shown to be highly effective in both instigating psychological stress given its social evaluative and uncontrollable nature (Dickerson & Kemedy, 2004) and inducing a cortisol response, with a two to threefold rise in salivary cortisol levels experienced by 70 to 80% of participants (Kudielka, Hellhammer, & Kirschbaum, 2007).

Life Experience Survey (LES)

The original version of the LES was used in this study (Sarason, et al., 1978). The LES is a questionnaire designed to measure life changes (also labelled as stressors) and their subjective impact. It comprises 47 items that list various common stressors. The participant identifies each event that has occurred during the last year as well as the perceived impact associated with that event at the time of occurrence. The *Impact score* is noted on a 7 point Likert scale in which the research participant can indicate the extent to which the event had either a positive impact (1, 2 or 3), a negative impact (-1, -2, -3) or "no impact" (0). Six total scores were derived from this questionnaire for the current study: 1) the total number of stressful life events, 2) the total number of positive events, 3) the total number of negative events, 4) the sum of the *Impact score* given to positive events (*Positive Impact Score*), 5) the sum of the *Impact score* given to

negative events (*Negative Impact Score*), and 6) the sum of both positive and negative impact scores (*Total Impact Score*).

Socio-demographic questionnaire

The socio-demographic questionnaire was related to general history, health history, and habits that may influence cortisol concentrations (e.g. sleep quality, alcohol consumption, nicotine use, medication and physical exercise). Questions about breast cancer history were added for women in the relevant group (e.g. time since diagnosis, age at diagnosis, initial cancer stage, and type of cancer treatment).

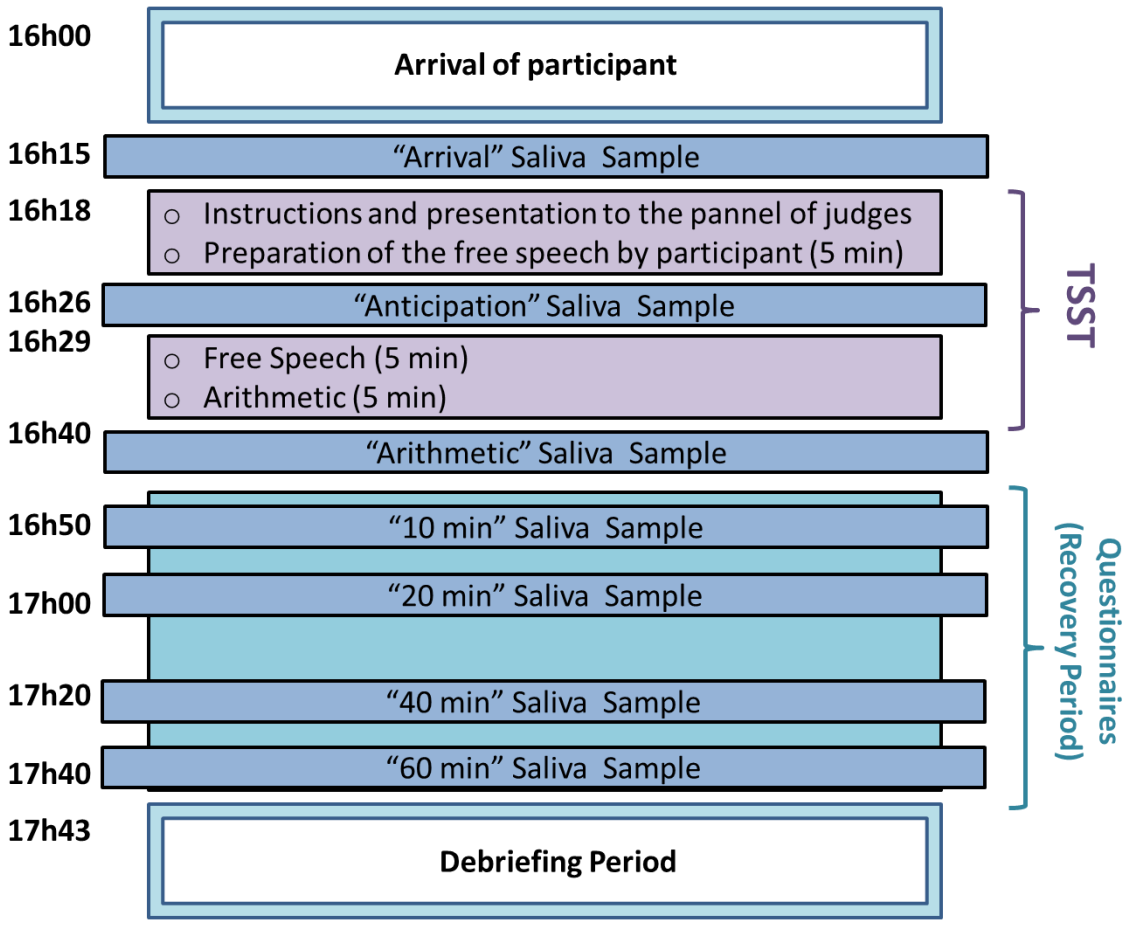
Salivary Cortisol

Samples were collected from oral swabs (Salimetrics, State College, PA) placed under the tongue for three minutes. Upon completion of the laboratory test, the saliva samples were centrifuged and transferred into Eppendorf tubes (1.5 mL). The tubes were stored in a -80 °C freezer for a maximum of six months. On the day of the analysis, samples were defrosted at room temperature and the concentration of salivary cortisol extracted from each saliva sample using enzyme immunoassay kits and the protocol designed by Salimetrics, State College, PA. Each saliva sample was assayed in duplicate.

Procedure

A more detailed description of the research protocol has been reported in Couture-Lalande and collaborators (2014). The sequence of steps in the Trier Social Stress Test protocol is presented in Figure 1.

Figure 1.



Participants who met eligibility criteria were invited to attend two laboratory visits at the University of Ottawa. During the first visit, participants were provided the consent form and details of the study. During the second visit, the TSST was carried out and saliva samples collected. The second laboratory visit was scheduled at the end of the day, (starting between 15h00 and 17h00), and lasted approximately 2 hours. Testing was done in the afternoon, the time of day corresponding to the lowest levels of cortisol concentrations in diurnal rhythms (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014). The first saliva sample was collected 10 minutes after arrival of the participants and again following the anticipatory period of the TSST (preparation of

the speech task) and immediately after the arithmetic task. Afterwards, participants were requested to complete a series of questionnaires, including the LES and socio-demographic questionnaires and to relax for one hour during which four additional saliva samples were obtained, at 10, 20, 40, and 60 minutes. Finally there was a debriefing period that gave the participant an opportunity to discuss the TSST and share her experience.

Results

Participants

Demographic characteristics

Nineteen breast cancer survivors and 17 women without a history of breast cancer completed the TSST as well as the LES. A complete list of the demographic characteristics of participants is shown in Table 1. The majority of participants were Caucasian, in a relationship, had completed an undergraduate degree, and in a white collar profession or retired. There was no significant group difference in mean age (t = -0.376, p = 0.709). The frequency of women with a post-menopausal status was also similar in both groups (x^2 = 0.509, p = 0.475).

Table 1. **Demographic characteristics of the breast cancer and control groups**

Demographic characteristics	Breast cancer survivors N = 19	Control group N = 17
Mean age ± SD (yr)	59 ± 11	58 ± 10
Age range (yr)	39 - 81	41 - 71
	%	**%**
Post-menopausal status	78	67
Ethnicity		
Caucasian	90	88
Black	---	6
Asian	---	6
First Nations	10	---
Marital status		
Single	11	6
Married/civil union/common law	63	53
Separated/divorced	21	29
Widowed	5	12
Highest level of education		
High school	32	35
College	21	23
Bachelor's degree	42	18
Master's degree	5	18
Doctoral degree	---	6
Employment status		
Blue collar	---	12
White collar	47	23
Self-employed	5	12
Homemaker	16	---
Medical leave of absence	---	6
Retired	32	47
Family Income		
Under $40,000	18	27
$40,000-99,999	64	46
$100,000 and above	18	27

Medical characteristics

The medical characteristics of the breast cancer survivors are presented in Table 2. Two women in the breast cancer group had experienced a recurrence in breast cancer prior to the study; one women reported one recurrence and the other, two recurrences. The information in Table 2 is based on the most recent breast cancer diagnosis of these participants.

Table 2. **Medical characteristics of the breast cancer group**

Medical characteristics	Breast cancer survivors N = 19
Mean age at diagnosis ± SD (yr)	55 ± 9
Age range (yr)	36 – 76
Mean time since diagnosis ± SD (yr)	4.4 ± 3
Time since diagnosis range (yr)	1.2 – 11.8
	%
Breast cancer stage	
0	21
1	42
2	21
3	16
Type of surgery	
Unilateral mastectomy	32
Bilateral mastectomy	36
Lumpectomy	32
Chemotherapy treatment	
Yes	58
No	42
Hormone therapy	
Yes	63
No	37
Radiation therapy	
Yes	58
No	42

Frequencies and perceived impact of stressful life events

The frequency of stressful life events that occurred during the last year is presented in Figure 2A. Breast cancer survivors reported an average of 5.7 stressful life events (SD = 3.91; total number of events ranged from 0 to 18) while women without a history of cancer reported an average of 5.4 stressful life events (SD = 4.76; total number of events ranged from 1 to 21). There were no significant group differences in the total number of stressful life events endorsed by breast cancer survivors and women without a history of cancer (t = -.266, p = .792). Furthermore, breast cancer survivors and women in the control group reported a similar number of positive events (t = -.769, p = .447), negative events (t = .150, p = .882) and events described as having had no impact (t = -.361, p = .720). Both groups reported more negative than positive events. In breast cancer survivors, on average, 60.5% of stressful life events were rated as negative, 34.0% as positive, and 5.5% as having had no impact. In the control group, 68.0% of stressful events were rated negatively, 27.5% positively, and 4.5% were rated as having had no impact. The frequency of positive and negative events was not significantly different in both breast cancer survivors (x^2 = 43.94; p = .143) and women in the control group (x^2 = 40.75; p = .91).

The impact scores, positive, negative, and total, are presented in Figure 2B. In summary, there were no significant differences between the perceived impact of events rated positively (t = -1.183; p = .245), negatively (t = .201; p = .842), as well as the total number of events that occurred during the last year (t = -.298; p = .767) in breast cancer survivors and women without a history of cancer.

Figure 2A

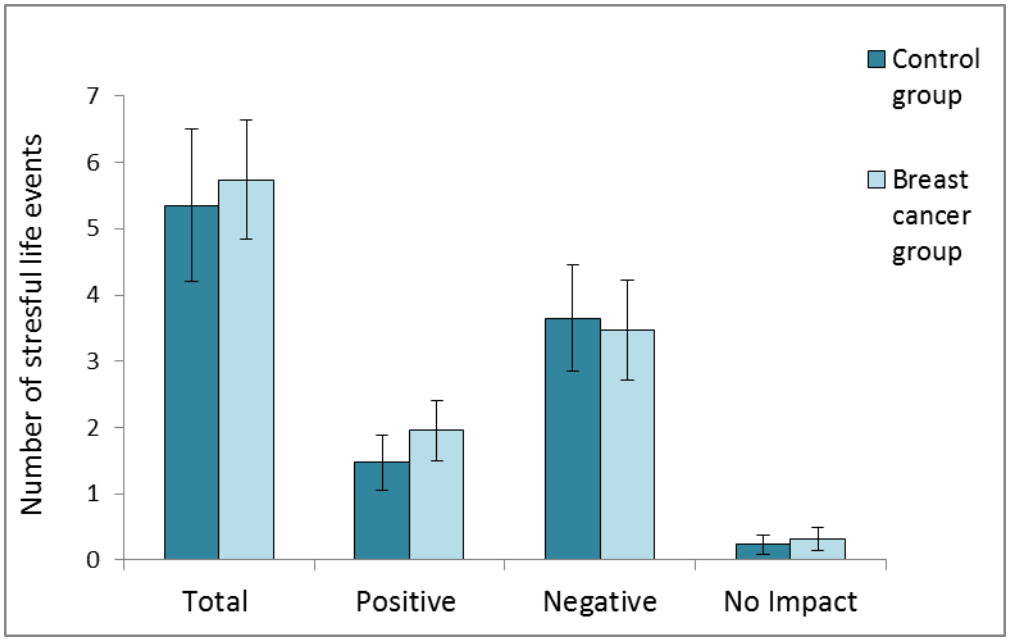
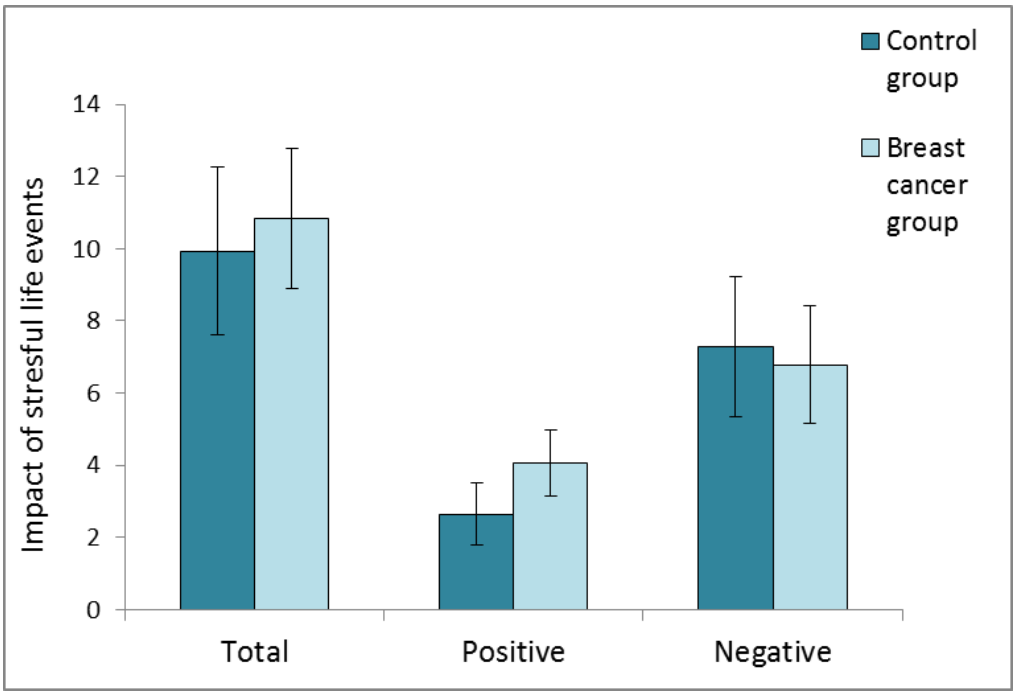


Figure 2B



Relationship between stressful life events and cortisol concentrations

Simple regression analyses were performed to evaluate the role of stressful life events that occurred during the last year on the peak cortisol concentration (average cortisol concentration observed at 10 and 20 minutes following the TSST). This is the time point at which the highest cortisol levels are consistently observed across participants. The number of positive, negative, and total number of stressful life events indicated by participants during the last year as well as their perceived impact were analysed separately.

Breast cancer survivors

In breast cancer survivors, there was a significant negative correlation between the number of stressful life events and the peak cortisol concentration (r = -.464, p = .035), indicating that a higher frequency of stressful events predicted lower levels of cortisol. Furthermore, the frequency of all stressful events accounted for 21.5% of the peak cortisol concentration (R^2 = .215); this relationship approached significance (F = 3.842; p = .070), likely due to the relatively small number of subjects and restricted range of stressful life events. Neither the negative nor positive stressful life events correlated with the peak cortisol concentration in breast cancer survivors (r = -.327, p = .108 and r = -.412, p = .057 respectively). Thus the valence, positive or negative, associated with stressful events did not significantly affect the peak cortisol concentrations compared to that of the overall frequency of stressful events in this group.

Women without a history of cancer

Compared to breast cancer survivors, no significant correlations were found between the total, positive, and negative frequencies of all stressful life events and the cortisol concentration at peak in this group (total: r = -.020, p = .474; negative: r = -.042; p = .446; positive: r = -.174, p = .285). Taken together, these results suggest that peak cortisol concentration are not impacted

by the overall or separate negative and positive frequencies in women without a history of breast cancer.

Impact of stressful life events and cortisol concentration

Breast cancer survivors

In breast cancer survivors, the perceived impact of stressful life events and peak cortisol concentrations was negatively correlated (r = -.552, p = .013); thus higher levels of perceived disturbance associated with stressful events predicted lower levels of peak cortisol. The perceived impact of all stressful events accounted for 30.5% of the peak cortisol concentration (R^2 = .305) and resulted in a significant effect (F = 6.145, p = .027). Positive but not negatively perceived stressful events were correlated with peak cortisol levels (r = -.455, p = .038 versus r = -.402, p = .062). In the case of positive events, the ANOVA approached significance (F = 3.663, p = .076) and accounted for 20.7% of the relationship (R^2 = .207).

Women without a history of cancer

None of the relationships examined between total, negative, or positively perceived impact of stressful life events and peak cortisol concentrations were found to be significant in this group (total: r = -.135, p = .330; positive: r = -.138, p = .326; negative: r = -.062, p = .420)).

Discussion

In our previous study, breast cancer survivors presented with lower levels of cortisol following the TSST compared to that of women without a history of breast cancer who were associated with typical patterns of reactive stress responses (Couture-Lalande et al., 2014). In this study, we were interested in understanding the role of stressful life events and their perceived impact on the cortisol secretion patterns of breast cancer survivors in the context of an acute stressor. As a first step, we recorded the number of stressful life events and their perceived

impact experienced by our sample of long-term breast cancer survivors and women without a history of cancer.

The number of stressful life events reported by our sample of breast cancer survivors was roughly twice that reported (5.7 versus 2.8) by newly diagnosed breast cancer patients in Lehto and collaborators' study (2008). Our sample reported a larger number of positive events while the number of negative events was comparable. It is possible that newly diagnosed breast cancer survivors are more affected by stressors related to their cancer diagnosis and thus less preoccupied by non-cancer life stress. In contrast, long-term breast cancer survivors may have preoccupations or - life stresses - similar to that of the rest of the population. For example, in a younger group of individuals, all students, Sarason and colleagues (1977) reported a frequency of stressful life events similar to that of our sample of long-term breast cancer survivors, suggesting that age and life stage may be even more closely related to the number of stressful life events than cancer stage.

Our results indicate no significant group differences in the overall frequency and perceived impact reported by breast cancer survivors and women without a history of cancer. In other words, non-cancer related stressful life events are not more prevalent in breast cancer survivors compared to that of women without a history of cancer. The perceived impact related to their stressful life events is also comparable suggesting an absence of cognitive bias in the appraisal of stressful life events in breast cancer survivors.

Breast cancer survivors who experienced higher number of stressful life events and perceived higher levels of disturbance associated with these events had lower levels of cortisol at peak. Our data suggest that stressful life events contribute to the low levels of cortisol concentration observed in some samples of breast cancer survivors (Andreano et al., 2012; Porter

et al., 2003; Spiegel et al., 2006; van der Pompe et al., 1996). Furthermore, the negative association between stressful life events experienced during a full year and cortisol levels support the hypothesis that hypocortisolism is associated with periods of chronic stress (Fries, et al., 2005; Kudielka et al., 2006; Kudielka, et al., 2009; Kudielka, et al., 2010; Miller, et al., 2007). However, the number of stressful life events and the perceived impact only explains part of the variance associated with reduced cortisol levels, suggesting that other components of the cancer experience may account for this difference.

In our previous study (Couture-Lalande et al., 2014), we reported that time since diagnosis was a medical variable significantly associated with lower levels of cortisol concentration following an acute stressor in these women. In addition, it is likely that other physiological mechanisms related to the tumor and its treatment may have had a role in the development of hypocortisolism. For example, high levels of proinflammatory cytokines that can be produced in the presence of a tumor or tumor cells have been associated with lower levels of cortisol (Schrepf, et al., 2013). Thus, the number of stressful life events and their perceived impact should be considered as one of the many factors that contribute to the atypical secretion patterns of cortisol reported in the population of breast cancer survivors.

Another important element of the present results is the negative relationship noted between the impact of positive stressful life events and levels of cortisol at peak. In most past studies, only negative stressful life events were associated with negative psychological outcome (Kornblith et al., 2001; Kornblith et al., 2011, 2003; Lehto et al., 2008) or with alteration of cortisol secretion (Armbruster et al., 2011; Wong, et al., 2012). Positive life events have been previously linked to a lower baseline level of cortisol after awakening. Some investigators interpret this as a favourable outcome (Pluess et al., 2012). However, our results suggest that life

events that are interpreted positively can also have an impact on the physiological stress response, which is consistent with Seyle's (1976) original definition that "stress is the nonspecific response of the body to any demand". In that framework, significant demands or changes, regardless of their positive or negative valence, would have the potential to create a stress response. For example, it is possible that a major life event such as winning the lottery, although positive in nature, could create a level of excitation that would result in activation of the HPA axis. These results suggest that positive and negative stressful life events are to be taken into account in the comprehension of atypical secretion patterns of cortisol in breast cancer survivors.

Furthermore, it is noteworthy that a higher number of stressful life events and their perceived impact were not related to lower or higher levels of cortisol following the acute stressor in women without a history of cancer. One explanation is that breast cancer survivors may be more sensitive to additional stress following a prolonged period of psychological stress related to their cancer diagnosis and\or related to the physiological changes associated with cancer and its treatment. Even though some women in the control group reported higher levels of stressful life events and high levels of disturbance associated with these events, these may not be enough to interfere with an HPA axis that is not challenged. Overall, these results emphasise the importance of monitoring the presence of stressful changes in breast cancer survivors long after diagnosis given its association with atypical cortisol secretion patterns.

We acknowledge that our results are limited by the small number of participants and small range of stressful life events reported by research participants. In addition to boosting the sample size, it would be useful to compare the effects of different indicators of psychosocial

stress on the cortisol secretion patterns of breast cancer survivors, in order to better understand the variables implicated in alteration of HPA axis functioning.

Finally, although the frequency of stressful life events experienced by an individual cannot be changed, relaxation training such as biofeedback has been shown to reduce the levels of anxiety in individual experiencing high levels of stressful life events (Weinman, Semchuk, Gaebe, & Mathew, 1983). Similarly, other programs that incorporate mindfulness training have been shown to significantly diminish levels of anxiety (Cramer, Lauche, Paul, & Dobos, 2012; Eyles et al., 2015; Hendersen, Massion, Clemow, Hurley, Druker, & Hébert; 2013), levels of distress (Baniasadi, Kashani, & Jamshidifar, 2014) as well as stress-related symptoms and post-traumatic symptoms (Tamagwa, Giese-Davis, Speca, Doll, Stephen, & Carlson, 2012) in breast cancer survivors. These may be useful strategies for diminishing the adverse effect of psychological stress associated with stressful life events and the experience of cancer and perhaps minimizing the alteration in cortisol secretion patterns.

General Discussion

The aim of this thesis was to unveil the extent to which breast cancer survivors are at risk of presenting dysregulated patterns of cortisol secretion. The role of stressful life events on this process was also analysed to investigate if the accumulation of stressors is one of the factors that interferes with the cortisol secretion rhythms of breast cancer survivors.

The cortisol activity of breast cancer survivors

The diurnal rhythms

One of the important findings of this thesis is that while a significant blunted cortisol stress response was observed in breast cancer survivors following an acute stressor, their diurnal cortisol rhythms were the same as those of women without a history of cancer. Normal diurnal rhythms have been described in other populations of newly diagnosed and early-staged breast cancer patients (Carlson, et al., 2007; Vedhara, et al., 2006) as well as in chronically stressed individuals (van der Hal-Van Raalte, Bakermans-Kranenburg, VanIjzendoorn, 2008). In contrast, flatter patterns of cortisol following an acute stressor appear to be more frequent in breast cancer patients (Andreano et al., 2012; Bower, Ganz, & Aziz, 2005) and individuals who have undergone a period of chronic stress (Carpenter, et al., 2011; Juster, et al., 2011; Gordis, et al., 2008; Gunnar, et al., 2009; Macmillan, et al., 2009; Matthews, et al., 2001; Rohler, et al., 2004; Trickett, et al., 2014).

Although altered patterns of diurnal cortisol have been reported in the past in long-term breast cancer survivors (Porter et al., 2003) and chronically stressed individuals (Dettenborn, et al., 2005), it appears that diurnal cortisol rhythms have a tendency to be more robust to external and internal changes in comparison to the cortisol stress response. For example, in a sample of holocaust survivors experiencing symptoms of post-traumatic stress disorder, dysregulation of

the HPA axis activity was only visible in the face of an acute stressor, while normal cortisol diurnal rhythms were observed (van der Hal-Van Raalte et al., 2008). In that regard, Kudielka and Wust (2010) have suggested that subtle dysregulations of the HPA axis may only be observable when the stress system is challenged. At a physiological level, it is noteworthy that virtually all organisms, from human to cyanobacteria, are highly influenced by the day/night cycles (Chung, Son, & Kim, 2011; Nader, Chrousos, & Kino, 2010). Thus, through evolution, organisms have developed a robust and sophisticated timekeeping system that exert endogenous circadian rhythms following the influence of light/dark information (Chung, et al., 2011; Nader, et al., 2010; Kassi & Chrousos, 2013). In humans, strong and predictable daily physiological and behavioural rhythms with nearly all body functions are observed, including sleep, body temperature, growth hormone, cortisol, etc. (Chan & Debono, 2010). The internal circadian rhythms impact greatly the healthy functioning of the organism by ensuring that certain physiological processes arise in coordination with others (Chung et al., 2011). It is possible that the mechanism underlying diurnal cortisol rhythmicity is more resilient and faithful to its baseline pattern following a context of adversity in comparison to the cortisol stress response.

Furthermore, in metastatic breast cancer patients, disease progression, disease severity, and even shorter survival time have been associated with dysregulated cortisol diurnal rhythms while no such strong associations have been raised in the context of the cortisol stress response (Abercrombie et al., 2004; Sephton et al., 2000; Touitou, et al., 1995). For example, in Sephton and colleagues' study (2000), flatter or abnormal cortisol diurnal rhythms predicted earlier mortality up to seven years later in metastatic breast cancer patients. Thus, the preservation of the diurnal rhythms seen in our sample of breast cancer survivors augurs well and can be interpreted as a sign of recovery.

The stress response

At the same time, the flat cortisol stress response profile observed in our sample of breast cancer survivors indicates that a subtle dysregulation of the HPA axis persists several years after diagnosis. Albeit less concerning than a dysregulated diurnal cortisol rhythm, a blunted cortisol stress response has been associated with both deleterious behavioural and physiological consequences for the organism (Galatzer-Levy et al., 2014; Bower, Ganz, Aziz, Olmstead, Irwin, & Cole, 2007; Burke, Fernald, Gertler, & Adler, 2005; Buske-Kirschbaum, Jobst, Wustmans, Kirschbaum, Rauh, & Hellhammer, 1997; Buske-Kirschbaum, von Auer, Krieger, Weis, Rauh, & Hellhammer, 2003).

The behavioural consequences. The stress response is part of the normal physiological regulation of the organism. Although it is often pictured in a negative light, a stress reaction is a very useful and adaptive mechanism. Thus, the stress response can be seen as an evolutionary ability of the organism to manage difficult situations through higher vigilance, arousal, and actions (Thiel and Dretsch, 2011). As mentioned by Thiel and Dretsch (2011), the absence of a reaction to a stressor can be detrimental in the sense that it would result in the inability for the organism to effectively deal with an external demand. The purpose of the stress reaction is to mobilize energy for the organism while inhibiting nonessential function. Throughout the release of cortisol, the HPA axis promotes energy metabolism, stress responsiveness, and information processing (van Praag, de Kloet, & van Os, 2004). It also prepares the organism for future needs by disposition of glycogen (van Praag, et al., 2004).

It has been suggested that a blunted cortisol stress response would be associated with weaker real life stress management (Burke, et al., 2005; Galatzer-Levy, et al., 2014). For instance, in Galatzer-Levy and colleagues' study (2014), the officer recruits who presented with

a blunted cortisol stress response following a laboratory stressor at the beginning of their training showed increasing levels of distress as well as a lack of resilience during the four years of their active duty. In contrast, the officer recruits who displayed a typical cortisol stress response manifested a pattern of adaptation through the same years. In another study by Burke and colleagues (2005), women from low social-economic status that exhibited a blunted cortisol slope in response to a naturalistic stressor displayed high levels of depressive symptomatology. Thus, as suggested by Galatzer-Levy, and colleagues (2014), the inability for an organism to mount a cortisol stress response following a stressor can result in weaker adaptation in response to stress.

In that context, it is possible that breast cancer survivors are at risk of experiencing more distress and weaker stress management skills when faced with a stressful situation given their attenuated physiological response to stress. Difficulties in managing real-life stressors could have important clinical implications for breast cancer survivors and should be investigated more thoroughly in future studies.

The physiological consequences. On the physiological side, low levels of cortisol following a stressful situation can have negative implications on the equilibrium of the body, in particular by contributing to higher and prolonged inflammatory response to stress (Bower et al., 2007; Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, Steptoe, 2003; McEwen et al., 1997). Several studies have reported that acute psychological stress leads to increased proinflammatory cytokine production (Maes et al., 1998; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001) and that glucocorticoids play an important role in containing the inflammatory response (Kunz-Ebrecht, et al., 2003; McEwen et al., 1997). Thus, the reduced availability of glucocorticoids in response to stress is suggested to weaken their

counterregulatory effect on proinflammatory cytokine leading eventually to inflammatory conditions (Bower et al., 2007; Kunz-Ebrecht, et al., 2003; McEwen et al., 1997). For example, Bower and colleagues (2007) documented the endocrine and immune response of fatigued breast cancer survivors following their participation in the TSST. They found that a reduced cortisol stress response was associated with enhanced inflammation, as measured by altered patterns of interleukin-1β and interleukin-6 production. The authors interpreted their results as an indication that the elevated inflammatory process seen in fatigued breast cancer survivors originated, at least in part, from their decreased cortisol response to stress.

In addition to fatigue, low levels of cortisol have also been associated with inflammatory conditions such as allergic asthma and atopic dermatitis (Buske-Kirschbaum, et al., 1997; Buske-Kirschbaum, et al., 2003; Sternberg, 2001). A hyporesponsive HPA axis has been suggested to increase the susceptibility for the host to develop chronic inflammatory disorders and/or may lead to aggravation of inflammatory disease (Buske-Kirschbaum, et al., 1997; Buske-Kirschbaum, et al., 2003). These results suggest that breast cancer survivors, especially early on, may be more at risk of developing inflammatory symptoms due to their low cortisol stress response. This would be particularly detrimental to breast cancer survivors who already suffer from chronic inflammatory disorders and may therefore be more vulnerable to experiencing a flare-up of their symptoms during stressful periods.

To summarize, while the absence of dysregulated diurnal rhythms can be interpreted as a sign of a healthy stress mechanism, the attenuated reactive cortisol response suggests that a subtle imbalance may be present in women with a history of breast cancer. While the experience of cancer encompasses many different factors that may play a role in regulating stress responses, the impact of the cumulative effect of stress was investigated more closely in this study.

The cumulative effect of stress and cortisol activity

The second important finding of this thesis is related to the impact of the cumulative stressful life events that occurred during the last year on the blunted cortisol stress response of breast cancer survivors. It was suggested several decades ago by Hans Seyle (1978) that the functioning of the stress system is optimal during punctual rather than chronic stress situations and that persistent activation of the physiological stress system could lead to the "wear and tear" of the body. Sterling and Eyer (1988) deepen that premise by examining the physiological basis linking a situation of chronic stress to disease processes. They suggested that under a situation of chronic stress, the different parameters of the organism would be required to fluctuate within broader range to appropriately match the demand of the environment. They coined the concept of *allostasis* which they define as "stability through change" and described *allostatic load* as the hidden cost of the body related to the constant ups and downs of the physiological stress response in situations of chronic stress. Subsequently, McEwens and collaborators emphasised the role of the HPA axis as one of the most common allostatic mediators buffering the effect of stress to avoid possible subsequent pathological states (McEwens, 1998; McEwens & Stellar, 1993). Different altered illustration of allostatic response have been proposed, one of those being a flatter stress pattern (McEwen, 1998), which is consistent with the blunted cortisol stress response displayed by our sample of breast cancer survivors. Thus, our results agree with the *allostatic load* framework which suggests that chronic stress can contribute to the dysregulation of the HPA axis activity over time. Furthermore, our data also support the hypocortisolism hypothesis given that the accumulation of stressors in our sample of breast cancer survivors was associated with lower levels of cortisol in response to a stressful situation.

The cumulative effect of stressful life events and their perceived impact is thus one of the factors that explain the blunted cortisol stress response presented by our sample of breast cancer survivors. However, it is noteworthy that the cumulative effect of stress only contributed for part of the variance associated with the reduced cortisol, suggesting that other components of the cancer experience may account for this difference.

Alternative physiological mechanisms

While the experience of cancer encompasses a number of different physical and psychological stressors for the organism, it is possible that the blunted cortisol stress response is the result of more than one mechanism. For instance, it has been suggested that an important feedback loop exists between proinflammatory cytokines and glucocorticoids. Specifically, it has been shown that while glucocorticoids negatively control cytokine production, interleukin-1 and interlukin-6 contribute to the activation of the HPA axis (Kunz-Ebrecht, et al., 2003). In the context of cancer, it is suggested that the presence of a tumor or tumor cells enhance the secretion of inflammatory cytokine interlukin-6 which in turn catalyze the release of glucocorticoids (Schrepf et al., 2013). Similar to the hypocortisolism hypothesis in the context of chronic stress, it is possible that the excessive cortisol production enhanced by proinflammatory cytokines diminishes with the passage of time as a way for the body to compensate the hypersecretion of cortisol, leading eventually to lower levels of cortisol. Thus, the blunted cortisol stress response measured in breast cancer survivors may be resulting in part from the chronic stress associated with the experience of cancer and also from the physiological reaction associated with the presence of malignancy. An interesting research avenue would be to explore the respective contribution of proinflammatory cytokines and psychological stress to the blunted cortisol response seen in breast cancer survivors. The measure of psychological and

physiological pathway of alteration of the HPA axis activity in the context of cancer could also lead to more effective multidisciplinary treatments.

Limitations and future directions

The findings presented in this thesis provide novel insight into the long term consequences of cancer on the HPA axis activity as well as the role of the cumulative effect of stress on this process. However, several limitations must be considered in interpreting these results. To begin, because convenience sampling was conducted, the participants in this study may not adequately represent all breast cancer survivors. The research participants were recruited locally, generally from urban areas, and were predominantly Caucasian and well-educated. In addition, the sample sizes of the sub-groups were small which limited further more resolved analyses. Breast cancer participants presented with a variety of cancer treatments - chemotherapy, radiation, hormonal therapy, or some combination of two or three of these treatments and it was not possible to conduct further analysis to determine if specific treatments were linked with increased cortisol dysregulation. Future studies should investigate the influence of treatment on HPA axis regulation in breast cancer survivors. Ideally, physical examinations and clinical assessments would be included such as endocrine tests to rule out factors that may affect HPA axis functioning.

Finally, another limitation of this thesis is the use of only one biomarker. Apart from the HPA axis, the sympathetic nervous system (SNS) is the other main component of the mammalian stress system. It is generally believed that the HPA axis and SNS interact with each other and buffer one another to preserve the stability of the organism during circadian activity as well as challenging situations (Figueiro & Rea, 2010). Thus, the inclusion of a biological marker of the SNS activity could provide a more complete portrait of the stress mechanisms in breast

cancer survivors. While traditional methods for assessing sympathetic activity are based on cardiovascular measures such as heart rate and blood pressure, the assessment of salivary norepinephrine has been suggested as a potential salivary indicator of the SNS activity (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). However, given that the transfer of norepinephrine from blood to saliva takes more than one hour, this biomarker seemed inadequate for the assessment of stress-induced changes in acute stress studies (Rohleder, et al., 2004). Chatterton, Vogelsong, Lu, Ellman, and Hudgens (1996) have suggested that the salivary protein alpha-amylase could be used as an index of the SNS. A study conducted later on by Rohleder and colleagues (2004) showed that the alpha-amylase activity increased following an acute stressor and that this enhancement was similar to the pattern seen in norepinephrine. Later on, more research suggested that alpha-amylase has a constant circadian rhythm and predicable secretion pattern following an acute stressor, reinforcing the status of salivary alpha-amylase as a reliable marker of the SNS activity (Nater & Rohleder, 2009; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Rohleder et al., 2004; Rohleder & Nater, 2009; Strahler, Berndt et al., 2010; Strahler, Mueller, Rosenloecher, Kirschbaum, & Rohleder, 2010). While, to our knowledge, the secretion patterns of alpha-amylase in breast cancer survivors have not been yet described in the literature, preliminary data from our laboratory suggest a tendency toward higher levels of alpha-amylase during the day as well as following a stressful situation in that population, corroborating the counterregulatory relationship between the SNS and the HPA axis.

Hair cortisol is another innovative method that could supplement the information obtained through salivary cortisol by providing a better appreciation of the retrospective cumulative cortisol concentration over longer periods of time. The analysis of hair cortisol as a method to measure chronic stress in humans has been examined in a growing number of

publications (Gow, Thomson, Rieder, Van Uum, & Koren, 2010; Meyer & Novak, 2012; Russel, Koren, Rieder, & Van Uum, 2012; Sharpley, McFarlane, & Slominski, 2012; Stalder & Kirchbaum, 2012; Stalder, Steudte, Miller, Skoluda, Dettenbord, & Kirschbaum, 2012). While salivary cortisol provides the real-time levels of psychological stress experienced by an individual, hair cortisol allow the measures of the integrated HPA axis activity over weeks and months (Meyer & Novak, 2012).

Clinical implications

The fact that stressful life events contribute to the blunted cortisol stress response of breast cancer survivors can lead to important clinical implications. It raises the importance of monitoring the presence of stressful changes in breast cancer survivors long after diagnosis. In that sense, physicians and health professionals working with the breast cancer population should be encouraged to document the presence of stressful life events and their subjective impact in breast cancer survivors as a part of their routine examinations. These results also provide additional importance to psychological treatments that can reduce the experience of stress. Since we know that the cumulative effect of stress is associated with abnormal cortisol stress patterns, treatments that reduce the experience of stress may accelerate the recovery of HPA activity in breast cancer survivors.

Relaxation and mindfulness techniques and cortisol activity. Given the association between psychological stress and dysregulated patterns of cortisol secretion in breast cancer survivors, an interesting avenue would be to determine if a psychological intervention, such as relaxation or mindfulness training, could normalize reactive cortisol responses in breast cancer survivors. For example, relaxation training such as biofeedback has been shown to reduce the levels of anxiety in individuals experiencing high levels of stressful life events (Weinman,

Semchuk, Gaebe, & Mathew, 1983). Similarly, other programs that incorporate mindfulness training have been shown to significantly diminish levels of anxiety (Cramer, Lauche, Paul, & Dobos, 2012; Eyles et al., 2015; Hendersen, Massion, Clemow, Hurley, Druker, & Hébert; 2013), levels of distress (Baniasadi, Kashani, & Jamshidifar, 2014) as well as stress related symptoms and post-traumatic symptoms (Tamagwa, Giese-Davis, Speca, Doll, Stephen, & Carlson, 2012) in breast cancer survivors. While no study has documented if psychological intervention, relaxation, or mindfulness training impact specifically the cortisol stress response of breast cancer survivors, changes toward more normalized cortisol diurnal rhythms (e.g. steeper slope) have been reported in some studies (Banasik, Williams, Haberman, Blank, & Bendel, 2011; Hsiao, et al., 2012). For example, in Hsiao and collaborators' study (2012), breast cancer survivors who completed eight weekly body-mind-spirit group therapy sessions (Chan, Ho, & Chow, 2001) showed a steeper cortisol diurnal rhythm in comparison to breast cancer survivors who only attended a single educational session. In contrast, these women displayed a flatter cortisol diurnal profile. In another study by Banasik and colleagues (2011), breast cancer survivors showed a progression from flatter to steeper cortisol diurnal rhythms following their participation in eight yoga class. These results suggest that psychological interventions and relaxing physical activity such as yoga can normalise the diurnal cortisol patterns of breast cancer survivors. Thus, psychological interventions, relaxation, and mindfulness training can prove to be helpful strategies for diminishing the levels of psychological stress and the cortisol rhythms alteration associated with the experience of cancer.

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Appendix A

Contributions of the collaborators

The conceptualisation and design of the two studies in this thesis was a collaborative effort between Dr. Bielajew and me. I was responsible for the research participant's recruitment and testing and benefited from the help of a team of research assistants and volunteers to perform the TSST. The laboratory procedure to extract the cortisol concentration from the saliva samples was performed by the laboratory technician Amira Mohamed. I was responsible for the data analysis, interpretation, and preparation of manuscripts with editing contributed by Dr. Bielajew. Consultations regarding statistical questions were handled by Drs. Schindler and Bielajew.

Appendix B

Informed consent

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Eligibility Criteria

Participants in this study include four groups of women:
- mothers with breast cancer
- non-mothers with breast cancer
- mothers without breast cancer
- non-mothers without breast cancer

You are eligible to participate if you fall in one of the four groups. The following exclusion criteria apply:
- Previous history of cancer (other than breast cancer)
- Previous history of chemotherapy or radiation (other than for breast cancer)
- Advanced disease (metastasis beyond axillary lymph nodes)
- Unstable psychiatric, neurological, or substance use disorders

Purpose of Study

Given that breast cancer survival rates have increased, it has become important to address the impact of breast cancer on psychosocial issues. The increased survival rate has made it important to focus not only on prevention and treatment, but to also consider issues related to coping and survival during diagnosis, treatment, and remission. One factor that has received little attention in this regard is motherhood. The purpose of this study is to determine whether being a mother with breast cancer affects stress as measured through stress biomarkers such as cortisol and whether cortisol levels are associated with self-reported measures of psychosocial functioning. Sustained elevated cortisol levels are linked to coronary artery disease which is now the leading cause of death in adult women. Understanding the factors that contribute to the long-term effects of stress in breast cancer survivors will have important consequences for the management of later health in these women.

Participation

Participation in this study involves three steps: 1) providing saliva samples over two consecutive days, 3) coming to the laboratory at the University of Ottawa to complete a brief psychological experiment, and 2) completing a set of questionnaires.

If you agree to participate in the study, the saliva collection tubes will be sent to you in the mail with detailed instructions on how and when to collect saliva samples over two consecutive days. Taking saliva samples involves putting a cotton-like roll under your tongue for 30 seconds and then placing the roll in a tube. You will be asked to provide saliva samples at home at waking, 30 minutes following waking, noon, 17h00, and 21h00 on two consecutive days.

You will then be asked to come to our laboratory at the University of Ottawa to perform a stress test, preferably 1 to 7 days after collecting the home saliva samples. You will also be asked to provide saliva samples before, during, immediately after the stress test, and 20, 40 and 60 minutes later. During the period following the test, you will be asked to complete several questionnaires. The questionnaires include sociodemographic information, medical history, and measures of psychosocial functioning (e.g. stress, social support, and physical health). Mothers with and without breast cancer will be asked to complete parenting questionnaires. The estimated duration of this laboratory visit is 1 hour, 30 minutes. Completion of the laboratory visit will mark the end of your participation in this study.

Risks

The only foreseeable physical risk in this study is tiredness while completing the battery of questionnaires. The questionnaires will take approximately 30 minutes to complete and participants are free to take breaks in between questionnaires if they please. Possible psychological or emotional risks include disclosure of sensitive medical information (e.g. breast cancer history), disclosure of personal information (e.g. parenting behaviour), anxiety or stress due to the nature of the questionnaires (e.g. experience with breast cancer), and anxiety or stress due to the stress test. If you feel any discomfort as a result of participating in this study, we recommend that you contact a distress center near you (e.g. in Ottawa, call The Distress Centre of Ottawa & Region, (613) 238-3311, http://www.dcottawa.on.ca). Here are some additional resources specific to women with breast cancer:

Canadian Breast Cancer Support Websites
Canadian Breast Cancer Foundation: http://www.cbcf.org/en-US/home.aspx
The Breast Cancer Society of Canada: http://www.bcsc.ca/
Canadian Breast Cancer Network: http://www.cbcn.ca/
Willow Breast Cancer Support Canada: http://www.willow.org/
Breast Cancer Action Ottawa: http://www.bcaott.ca/

Benefits

There is no direct benefit to participants in this study other than the knowledge that they have contributed to our understanding of how breast cancer affects various psychosocial factors and its relationship to parenting. If you wish, you will be given feedback about this at the end of the study.

Compensation

Compensation will be in the form of a draw of three cash prizes of $250 in September 2009, January 2010, and May 2010. In order to participate in the draw, you must provide us with your email address. You will also be given $12 to cover your transportation cost or the cost of a campus parking pass.

Questions, Withdrawal, and Confidentiality

Your participation in this study is entirely voluntary and, even if you decide to participate, you are free to withdraw at any time. Your decision to withdraw will not affect your chances of winning the cash prize.

The information collected from you as part of this study will be held in the strictest confidence. Completed questionnaires will be identified by number—your name will not appear on them. Any identifying or contact information will be kept separate from the completed questionnaires. All data will be stored in a locked laboratory on a computer that is password protected for five years, at which time they will be destroyed. Data from this study may be published but these data will not bear your name or any other identifying information.

If you have any questions or concerns about this research, or should you desire further explanation during the course of the study, you are encouraged to contact Dr. Catherine Bielajew, principal investigator.

Any information requests or complaints about the ethical conduct of the project may be addressed to the Social Sciences and Humanities Research Ethic Board or the University of Ottawa or by calling the Protocol Officer for Ethics in Research at (613) 562-5841 or emailing ethics@uottawa.ca.

Study Findings

The Principal Investigator, Dr. Catherine Bielajew, will send an email to all participants with a summary of findings.

Consent

I agree to participate in the present study entitled "Relationship between motherhood and breast cancer on perceived stress and the physiological stress response" that is being conducted by Dr. Catherine Bielajew, Dr. Sophie Lebel, Jacinthe Faucher, and Marie-Ève Couture-Lalande of the School of Psychology at the University of Ottawa. I have read and understand the information presented above regarding the purpose of the study; participation; risks and benefits; compensation; and questions, withdrawal and confidentiality.

I understand the above information and voluntarily consent to participate in this study.

Appendix C

Laboratory protocol and verbatim

Before the participant arrives:

Prepare the room where the TSST will happen:

- Have three chairs behind the table.
- Have 3 pens and 3 papers on the table (in front of each chair)
- Move the book shelf beside the table
- Place the camera on the bookshelf facing the X (plug in the camera, but do not record)

TSST script:

Please come with me to the next room (*Participants will then be escorted to room B and introduced to their next task. In room B, at least 2 people will be sitting in the room (confederates), and a video camera will be set up*).

Please stand here, in front of the selection committee and in front of the video camera. For your first task, I would like you to take over the role of a job applicant who has been invited for a personal interview with the selection committee (*researcher points to the committee sitting in the room*). I will be taking you back to the previous room and you will be given 5 minutes of preparation time. Following the preparation period you will come back into this room and will introduce yourself to the committee and perform a free speech of 5 minutes duration to convince the committee that you are the perfect applicant for the vacant position. The committee members have been specially trained to monitor nonverbal behaviour and during this task a video analysis of your performance will also be conducted (*Following these instructions, the participants will return to room A and will be given 5 minutes to prepare their speech. The participant will be provided with paper and pencil to outline their talks; however, they will not be allowed to use the written notes for their speech. you can say:* You can write whatever you want on this paper to prepare, but you will not be allowed to bring this paper to the other room when I come back to get you. *The experimenter will stand outside the room while they prepare*. (*After 5 minutes*)

Return to the room B and collect the saliva sample labeled «anticipatory» you can say: Before we go to the other room, I would like to receive another saliva sample from you. Remember to pretend to chew while taking the saliva sample. *The participant must put the swab under her tongue for 3 minutes exactly. While the participant takes the sample, she fills out the VAS. You can say:* I would like you to indicate on the visual analog scales how you currently feel *When the participant is done taking the sample, place the tube in the cooler and say:* Please follow me and I will take you back to the next room to perform your task (*The participant is escorted to room B*). Please deliver your speech (*The researcher will pretend to turn on the video camera*). (*If the participant finishes their speech in less than 5 minutes, the experimenter will respond*), "You still have some time left. Please continue!" (*Once the 5 minutes is over, the participant will then go on to the next task*). Now for your second task, please serially subtract the number 13 from 1,022 as fast and as accurately as possible. So basically, you take the number 1,022, subtract 13, and whatever answer you get, you subtract 13 again and so on. (*On*

every failure the subjects have to restart at 1,022 with the experimenter interfering) "Stop. Please start again at 1,022." (*After 5 minutes of the mental arithmetic task the participant will be taken back to room A*). Please follow me back to the next room. (*Upon arrival to the next room, collect the saliva sample labeled: «Arithmetic», say)* Before we continue, I would like to receive another saliva sample from you. Remember to pretend to chew while taking the saliva sample. *The participant must put the swab under her tongue for 3 minutes exactly. While the participant takes the sample, she fills out the VAS. You can say:* I would like you to indicate on the visual analog scales how you currently feel (*When the participant is done taking the sample, place the tube in the cooler When the participant is ready (<u>before</u> or after the end of this saliva sample) say:* Here is a battery of questionnaires for you to complete. (<u>Name of good cop here)</u> will keep coming back to collect more saliva, please remember to avoid drinking water until she/he tells you that it is a good time to do so. Also remember that we are not allowed to answer any questions about what happened until the end of the session.

<u>Particular situations, what happens if:</u>

- The participant tries to shake the committee members' hands. → Please stay on the X.
- The participant asks if we have any questions: → It's a free speech.
- The participant says that they do not want to continue. → DO NOT FORCE THEM TO CONTINUE, ask if they would like to try the second task (arithmetic), if so introduce the second task as you normally would: «For your second task,…» If they say no say: «that is ok (with no emotion), and ask them to follow you back into the other room, see if they don't mind completing the questionnaires, and take their saliva sample. Never let them leave without doing the debriefing with them.

Appendix D

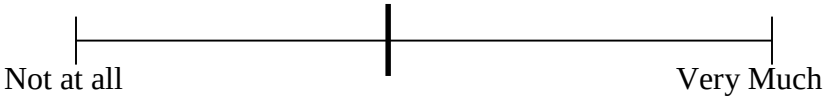
Participant ID:_____
Sample: Arrival

Visual Analog Scales

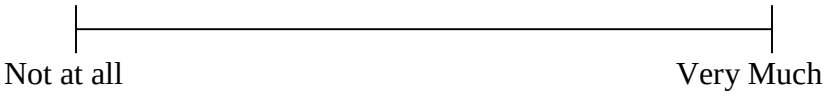
Below the example, please draw a vertical line on the horizontal line to indicate your feelings ***right at this present moment***.

Example:

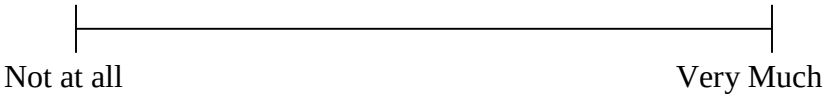
I feel happy.



I feel stressed.



I feel anxious.



Appendix E

SOCIO-DEMOGRAPHIC QUESTIONNAIRE

The following question involves gathering information with respect to your socio-demographic background. For each question, please circle the appropriate answer.

General history

sd1. What is your ethnic background?
1. White/Caucasian
2. Black (e.g. Haitian, African, Jamaican, Somali)
3. Asian (e.g. Chinese, East Indian, Japanese, Vietnamese)
4. Latino or Hispanic
5. Pacific Islander
6. Middle Eastern
7. Native Canadian/First Nations/Métis
8. Other. Specify: _____

sd2. How old are you? _____

sd3. What is your relationship status?
1. Single
2. Dating
3. Common Law
4. Married/Civil Union
5. Separated/Divorced
6. Widowed

sd4. If you are in a relationship, how long have you been with your partner (months and/or years)? _____

sd5. What level of education have you completed?
1. Elementary School
2. High School
3. College
4. Bachelor's Degree
5. Master's Degree
6. Doctoral Degree

sd6. What is your current work status?
1. Blue collar (construction, factory worker, manual work, etc.)
2. White collar (administrator, lawyer, director, office work, sales, etc.)
3. Business owner or self-worker
4. Unemployed
5. Student
6. Stay at home

7. Medical leave of absence
sd6a. If so, what was your employment before? _____
sd6b. How long have you been on a medical leave of absence? _____
8. Retired
sd6c. If so, what was your employment before? _____
9. Other
sd6d. Please specify: _____

sd7. What is your current annual family income?
1. Under $20, 000
2. $20, 000 – $39, 999
3. $40, 000 - $59, 999
4. $60, 000 – $79, 999
5. $80, 000 – $99, 999
6. $100, 000 – $119, 999
7. $120, 000 – $139, 999
8. $140, 000 – $159, 999
9. $160, 000 – $179, 999
10. $180, 000 – $199, 999
11. $200, 000 and above

Breast cancer history
The breast cancer history section is divided in two sections. The first one pertains to an initial breast cancer diagnosis and its treatment, the second section pertains to a recurrence in breast cancer (if this applies) and its treatment.

Initial breast cancer:
sd8. When were you diagnosed with your initial breast cancer? _____/_____
Month Year

sd9. How old were you when you were diagnosed? _____

sd10. What stage of breast cancer were you diagnosed with?
1. Stage 0 (very early or "in situ")
2. Stage I (localized, no spreading)
3. Stage II (some localized spreading into lymph nodes)
4. Stage III (some localized spreading into lymph nodes)
5. Stage IV (metastases, where the cancer has spread to other parts of the body)
6. Not sure

We would like to know more about the treatment you received for your initial breast cancer diagnosis.

sd11. What type of surgery did you have?
1. Unilateral mastectomy
2. Bilateral mastectomy
3. Lumpectomy on one breast
4. Lumpectomy on both breasts
5. Surgery

sd12. Did you receive chemotherapy?
1. Yes
2. No, but I will (please go directly to question no. 13)
3. No, and I will not (please go directly to question no. 13)

What type of chemotherapy did you receive?
sd12a. I received neoadjuvant chemotherapy only (given before surgery to shrink the size of a tumor)
1. Yes
2. No

sd12b. I received adjuvant chemotherapy only (given after surgery to reduce the risk of recurrence)
1. Yes
2. No

sd12c. I received palliative chemotherapy (used to control the cancer in settings in which the cancer has spread beyond the breast and localized lymph nodes)
1. Yes
2. No

What chemotherapy regimen did you receive?
sd12d. Frequency (e.g. once every three weeks): _____

sd12e. Duration (e.g. 5 months): _____

sd13. Did you receive hormone therapy (or are you still receiving hormone therapy)?
1. Yes
2. No, but I will (please go directly to question no. 14)
3. No, and I will not (please go directly to question no. 14)

Sd13a. What type of hormone therapy did you receive (i.e. Tamoxifen)? _____

Sd13b. How long did you receive hormone therapy for (or how long have you been receiving hormone therapy)? _____

sd14. Did you receive radiation therapy?
1. Yes
2. No, but I will (please go directly to question no. 15)
3. No, and I will not (please go directly to question no. 15)

sd14a. If yes, how many sessions in total did you have? _____

sd15. Have you had breast reconstruction surgery or are you planning on having this surgery?
1. Yes, I have had breast reconstruction surgery
2. Yes, I plan on having breast reconstruction surgery
3. No, I have not, and do not plan on having breast reconstruction surgery

sd16. Have you experienced a recurrence in breast cancer?
1. Yes
2. No (Please go directly to question no. 26)

Recurrence in breast cancer

sd17. When did your recurrence occur? _____/_____
Month Year

sd18. How old were you when you were diagnosed with your recurrence? _____

sd19. What stage of breast cancer were you diagnosed with for this cancer?
1. Stage 0 (very early or "in situ")
2. Stage I (localized, no spreading)
3. Stage II (some localized spreading into lymph nodes)
4. Stage III (some localized spreading into lymph nodes)
5. Stage IV (metastases, where the cancer has spread to other parts of the body)
6. Not sure

sd20. What type of surgery did you have?
1. Unilateral mastectomy
2. Bilateral mastectomy
3. Lumpectomy on one breast
4. Lumpectomy on both breasts
5. Surgery

sd21. Did you receive chemotherapy?
1. Yes
2. No (please go directly to question no. 22)
3. No, and I will not (please go directly to question no. 22)

What type of chemotherapy did you receive? (Please select all the ones that apply)
sd21a. I received neoadjuvant chemotherapy only (given before surgery to shrink the size of a tumor)
1. Yes
2. No

sd21b. I received adjuvant chemotherapy only (given after surgery to reduce the risk of recurrence)
1. Yes
2. No

sd21c. I received palliative chemotherapy (used to control the cancer in settings in which the cancer has spread beyond the breast and localized lymph nodes)
1. Yes
2. No

sd22. Did you receive hormone therapy (or are you still receiving hormone therapy)?
1. Yes
2. No, but I will (please go directly to question no. 23)
3. No, and I will not (please go directly to question no. 23)

sd22a. What type of hormone therapy did you receive (i.e. Tamoxifen)? _____

sd22b. How long did you receive hormone therapy for (or how long have you been receiving hormone therapy)? _____

sd23. Did you receive radiation therapy?
1. Yes
2. No, but I will (please go directly to question no. 24)
3. No, and I will not (please go directly to question no. 24)

sd23a. If yes, how many sessions in total did you have? _____

sd24. Have you had breast reconstruction surgery or are you planning on having this surgery?
1. Yes, I have had breast reconstruction surgery
2. Yes, I plan on having breast reconstruction surgery
3. No, I have not, and do not plan on having breast reconstruction surgery
sd25. Have you experienced a recurrence in breast cancer?
1. Yes (Please complete the “recurrence in breast cancer” section)
2. No (Please skip to the “other health history” section)

Other health history

sd26. Have you been diagnosed with another cancer (of any type) after being diagnosed with breast cancer (apart from breast cancer recurrence)?
1. Yes
2. No (if no, please go directly to question no. 27)

sd26a. What kind of cancer were you diagnosed with? _____

sd26b. When were you diagnosed? _____

sd26c. How was it treated? Please indicate whether you had surgery, chemotherapy, hormone therapy, radiation therapy, or another treatment:
_____

sd27. Have you ever had a chronic medical condition other than breast cancer (e.g. diabetes, high blood pressure, multiple sclerosis, etc.)?
1. Yes. Please specify: _____
2. No (if no, please go to question no. 28)

sd27a. How much do you worry about this (these) medical condition(s)?
1. Not at all
2. A little bit
3. A lot
4. All the time

sd27b. Does (Do) this (these) medical condition(s) interfere with your daily activities?
1. Not at all
2. A little bit
3. A lot
4. All the time

The following questions refer to your parenting history; if you do not have any children, please go directly to question no. 32

Habits

sd33. Do you take any prescribed medication? If so, please list the name(s) and dose(s):
_____
_____

sd34. Please indicate the average amount of alcoholic beverages you consume per day.
1. 0-1
2. 2-3
3. 4-5
4. 6-7
5. 8 +

sd35. Please indicate the average amount of caffeinated beverages you consume per day.
1. 0-1
2. 2-3
3. 4-5
4. 6-7
5. 8 +

sd36. Do you smoke cigarettes? _____
1. Yes
2. No (if no, please go directly to question no. 38)

sd37. If so, please indicate the average amount of cigarettes you smoke per day?
1. Half a pack or less
2. 1 pack
3. 1 ½ pack
4. 2 packs or more

sd38. How often do you brush your teeth?
1. Two times or more per day
2. One time per day
3. One time every two days
4. Less than one time every two days

sd39. When you brush your teeth, is there blood in your saliva?
1. Always
2. Often
3. Sometimes
4. Never

sd40. When was your last visit to the dentist?
1. Within the last 6 months
2. Within the last year
3. Within the last 18 months
4. Within the last 24 months
5. More than 24 months ago

sd41. How often do you do cardiovascular exercise?
1. Once a week or less
2. Two to three times a week
3. Four to five times a week
4. Six to seven times a week
5. More than seven times a week

Appendix F

BIDIMENSIONAL FATIGUE SCALE

Read each item carefully and indicate which comes closer to how you have been feeling in the past two weeks, do not take too long over your replies.

Better than usual	No more than usual	Worse than usual	Much worse than usual
1	2	3	4

BFS 1. Do you have problems with tiredness?	1	2	3	4
BFS 2. Do you need to rest more?	1	2	3	4
BFS 3. Do you feel sleepy or drowsy?	1	2	3	4
BFS 4. Do you have problems starting things?	1	2	3	4
BFS 5. Are you lacking in energy?	1	2	3	4
BFS 6. Do you have less strength in your muscles?	1	2	3	4
BFS 7. Do you feel weak?	1	2	3	4
BFS 8. Do you have difficulty concentrating?	1	2	3	4
BFS 9. Do you have problems thinking clearly?	1	2	3	4
BFS 10. Do you make slips of the tongue when speaking?	1	2	3	4
BFS 11. How is your memory?	1	2	3	4

Appendix G

THE LIFE EXPERIENCES SURVEY

Listed below are a number of events which sometimes bring about change in the lives of those who experience them and which necessitate social readjustment.

Please check those events which you have experienced in the recent past and indicate the time period during which you have experienced each event. Be sure that all check marks are directly across from the items they correspond to.

Also, for each item checked below, *please indicate the extent to which you viewed the event as having either a positive or negative impact on your life* at the time the event occurred.

Extremely negative -3	Moderately negative -2	Somewhat negative -1	No impact 0	Slightly positive +1	Moderately positive +2	Extremely positive +3

	0 month to 6 months	7 months to 1 year							
LES 1. Marriage	□	□	-3	-2	-1	0	+1	+2	+3
LES 2. Detention in jail or comparable institution	□	□	-3	-2	-1	0	+1	+2	+3
LES 3. Death of spouse	□	□	-3	-2	-1	0	+1	+2	+3
LES 4. Major change in sleeping habits (much more or much less sleep)	□	□	-3	-2	-1	0	+1	+2	+3
LES 5. Death of close family member:									
LES 5a. mother	□	□	-3	-2	-1	0	+1	+2	+3
LES 5b. father	□	□	-3	-2	-1	0	+1	+2	+3
LES 5c. brother	□	□	-3	-2	-1	0	+1	+2	+3
LES 5d. sister	□	□	-3	-2	-1	0	+1	+2	+3
LES 5e. grandmother	□	□	-3	-2	-1	0	+1	+2	+3
LES 5f. grandfather	□	□	-3	-2	-1	0	+1	+2	+3
LES 5g. other (specify)	□	□	-3	-2	-1	0	+1	+2	+3
LES 6. Major change in eating habits (much more or much less food intake)	□	□	-3	-2	-1	0	+1	+2	+3
LES 7. Foreclosure on mortgage or loan	□	□	-3	-2	-1	0	+1	+2	+3
LES 8. Death of close friend	□	□	-3	-2	-1	0	+1	+2	+3
LES 9. Outstanding personal achievement	□	□	-3	-2	-1	0	+1	+2	+3

LES 10. Minor law violations (traffic tickets, disturbing the peace, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 11. Pregnancy	□	□	-3	-2	-1	0	+1	+2	+3
LES 12. Changed work situation (different work responsibility, major change in working conditions, working hours, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 13. New job	□	□	-3	-2	-1	0	+1	+2	+3
LES 14. Serious illness or injury of close family member:									
LES 14a. father	□	□	-3	-2	-1	0	+1	+2	+3
LES 14b. mother	□	□	-3	-2	-1	0	+1	+2	+3
LES 14c. sister	□	□	-3	-2	-1	0	+1	+2	+3
LES 14d. brother	□	□	-3	-2	-1	0	+1	+2	+3
LES 14e. grandfather	□	□	-3	-2	-1	0	+1	+2	+3
LES 14f. grandmother	□	□	-3	-2	-1	0	+1	+2	+3
LES 14g. spouse	□	□	-3	-2	-1	0	+1	+2	+3
LES 14h. other (specify)	□	□	-3	-2	-1	0	+1	+2	+3
LES 15. Sexual difficulties	□	□	-3	-2	-1	0	+1	+2	+3
LES 16. Trouble with employer (in danger of losing job, being suspended, demoted, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 17. Trouble with in-laws	□	□	-3	-2	-1	0	+1	+2	+3
LES 18. Major change in financial status (a lot better off or a lot worse off)	□	□	-3	-2	-1	0	+1	+2	+3
LES 19. Major change in closeness of family members (increased or decreased closeness)	□	□	-3	-2	-1	0	+1	+2	+3
LES 20. Gaining a new family member (through birth, adoption, family member moving in, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 21.Change of residence	□	□	-3	-2	-1	0	+1	+2	+3
LES 22. Marital separation from mate (due to conflict)	□	□	-3	-2	-1	0	+1	+2	+3
LES 23. Major change in church activities (increased or decreased attendance)	□	□	-3	-2	-1	0	+1	+2	+3
LES 24. Marital reconciliation with mate	□	□	-3	-2	-1	0	+1	+2	+3
LES 25. Major change in number of arguments with spouse (a lot more or a lot less arguments)	□	□	-3	-2	-1	0	+1	+2	+3

LES 26. Change in husband's work outside the home (loss of job, beginning new job, retirement, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 27. Major change in usual type and/or amount of recreation	□	□	-3	-2	-1	0	+1	+2	+3
LES 28. Borrowing more than $10,000 (buying home, business, etc)	□	□	-3	-2	-1	0	+1	+2	+3
LES 29. Borrowing less than $10,000 (buying car, T.V., getting school loan, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 30. Being fired from job	□	□	-3	-2	-1	0	+1	+2	+3
LES 31. Having abortion	□	□	-3	-2	-1	0	+1	+2	+3
LES 32. Major personal illness or injury	□	□	-3	-2	-1	0	+1	+2	+3
LES 33. Major change in social activities, e.g., parties, movies, visiting (increased or decreased participation)	□	□	-3	-2	-1	0	+1	+2	+3
LES 34. Major change in living conditions of family (building new home, remodeling, deterioration of home, neighborhood, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 35. Divorce	□	□	-3	-2	-1	0	+1	+2	+3
LES 36. Serious injury or illness of close friend	□	□	-3	-2	-1	0	+1	+2	+3
LES 37. Retirement from work	□	□	-3	-2	-1	0	+1	+2	+3
LES 38. Son or daughter leaving home (due to marriage, college, etc.)	□	□	-3	-2	-1	0	+1	+2	+3
LES 39. Ending of formal schooling	□	□	-3	-2	-1	0	+1	+2	+3
LES 40. Separation from spouse (due to work, travel, etc)	□	□	-3	-2	-1	0	+1	+2	+3
LES 41. Engagement	□	□	-3	-2	-1	0	+1	+2	+3
LES 42. Breaking up with boyfriend/girlfriend	□	□	-3	-2	-1	0	+1	+2	+3
LES 43. Leaving home for the first time	□	□	-3	-2	-1	0	+1	+2	+3
LES 44. Reconciliation with boyfriend/girlfriend	□	□	-3	-2	-1	0	+1	+2	+3
Other recent experiences which have had an impact on your life. Please list and rate.									
LES 45. _____	□	□	-3	-2	-1	0	+1	+2	+3
LES 46. _____	□	□	-3	-2	-1	0	+1	+2	+3
LES 47. _____	□	□	-3	-2	-1	0	+1	+2	+3