"CENTRAL ADIPOSITY AND INCREASED CORTISOL REACTIVITY TO STRESS: POTENTIAL SOURCE OF INCREASED BREAST CANCER RISK AMONG WOMEN WITH A FAMILY HISTORY OF BREAST CANCER"

By

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DISSERTATION

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The following dissertation is my own work. Under the guidance of my mentors Dr. Dana H. Bovbjerg and Dr. Dirk H. Hellhammer, I was responsible for a) developing the idea for this work, b) conducting the literature research and composing the theoretical background, c) developing the hypotheses to be tested, d) creating the analysis plan, e) conducting all data analyses using the Statistical Package for the Social Sciences 11.0, f) presenting all data graphically and in written, and g) composing the discussion and conclusion of the findings.

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General Abstract

Objective: Only 20-25% of the variance for the two to four-fold increased risk of developing breast cancer among women with family histories of the disease can be explained by known gene mutations. Other factors must exist. Here, a familial breast cancer model is proposed in which overestimation of risk, general distress, and cancer-specific distress constitute the type of background stress sufficient to increase unrelated acute stress reactivity in women at familial risk for breast cancer. Furthermore, these stress reactions are thought to be associated with central adiposity, an independent well-established risk factor for breast cancer. Hence, stress through its hormonal correlates and possible associations with central adiposity may play a crucial role in the etiology of breast cancer in women at familial risk for the disease. *Methods:* Participants were 215[1] healthy working women with first-degree relatives diagnosed before (high familial risk) or after age 50 (low familial risk), or without breast cancer in first-degree relatives (no familial risk). Participants completed self-report measures of perceived lifetime breast cancer risk, intrusive thoughts and avoidance about breast cancer (Impact of Event Scale), negative affect (Profile of Mood States), and general distress (Brief Symptom Inventory). Anthropometric measurements were taken. Urine samples during work, home, and sleep were collected for assessment of cortisol responses in the naturalistic setting where work was conceptualized as the stressful time of the day. *Results:* A series of analyses indicated a gradient increase of cortisol levels in response to the work environment from no, low, to high familial risk of breast cancer. When adding breast

[1] or less, depending on the type of analysis

cancer intrusions to the model with familial risk status predicting work cortisol levels, significant intrusion effects emerged rendering the familial risk group non-significant. However, due to a lack of association between intrusions and cortisol in the low and high familial risk group separately, as well as a significant difference between low and high familial risk on intrusions, but not on work cortisol levels, full mediation of familial risk group effects on work cortisol by intrusions could not be established. A separate analysis indicated increased levels of central but not general adiposity in women at high familial risk of breast cancer compared to the low and no risk groups. There were no significant associations between central adiposity and cortisol excretion. *Conclusion:* A hyperactive hypothalamus-pituitary-adrenal axis with a more pronounced excretion of its end product cortisol, as well as elevated levels of central but not overall adiposity in women at high familial risk for breast cancer may indicate an increased health risk which expands beyond that of increased breast cancer risk for these women.

General Introduction

Breast cancer is the most frequently diagnosed malignancy among women in the Western world. It is the second leading cause of cancer mortality next to lung cancer in American women. Even though detection at earliest stages promises a 95% 5-year survival rate, 39,000 American women will die of the disease this year (ACS, 2004). The adverse side effects of the treatments for breast cancer, including disfiguring surgery, radiation and chemotherapy, are well-known among the general population. A national survey of 1045 women (Spittle & Morgan, 1999) revealed that breast cancer is one of the most feared of all diseases.

The increased risk of developing breast cancer among women with family histories of the disease has long been recognized (Pharoah et al., 2002). Healthy women with family histories that include even a single first-degree relative with breast cancer, have a significantly elevated risk of two to four times of developing the disease themselves compared to women without breast cancer in close relatives (Collaborative Group, 2001; Evans & Lalloo, 2002). Even though research on gene mutations to explain breast cancer risk has made revolutionary advances over the last decade (Dumitrescu & Cotarla, 2005) (for review), effects of all known gene mutations suspected to influence breast cancer risk have been estimated to account for only 20-25% of familial risk (Easton, 1999). Hence, other risk factors for explaining the increased susceptibility among women with family histories of breast cancer must exist.

Among one of the well established risk factors for breast cancer is central adiposity (characterized as excess fat around the waist), and more specifically central

adiposity adjusted for general obesity in premenopausal women (Harvie, Hooper, & Howell, 2003). Central adiposity adjusted for general obesity is a surrogate measure of visceral fat (Janssen, Heymsfield, Allison, Kotler, & Ross, 2002), a compartment of abdominal fat that is markedly different from subcutaneous and peripheral fat in its morphology and endocrine action (Pedersen, Jonler, & Richelsen, 1994; Jazet, Pijl, & Meinders, 2003; Cinti, 2001; Trayhurn & Beattie, 2001; Faloia, Camilloni, Giacchetti, & Mantero, 2000). It has greater blood flow and up to four times more glucocorticoid receptors than peripheral fat making it especially sensitive to the fat-accumulating effects of circulating cortisol. Cortisol is the end product of the hypothalamus-pituitary-adrenal (HPA) axis, one of the two major systems activated by stress next to the sympathoadrenal medullary system (SAM) (Nicolaidis, 2002). There is evidence of common familial components underlying cortisol and abdominal fat covariation caused by common polygenic determinants explaining 16% to 20% of the phenotypic variance (Feitosa et al., 2002).

Women with family histories of breast cancer have been characterized as having higher levels of abdominal fat (Schapira, Kumar, & Lyman, 1993), as well as demonstrating increased cortisol responses to stress in the laboratory (Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003). This increased cortisol reactivity to acute stress is consistent with findings of increased psychological distress (general and cancer-specific) in women with family histories of breast cancer (Baider, Ever-Hadani, & Kaplan De-Nour, 1999; Erblich, Montgomery, Valdimarsdottir, Cloitre, & Bovbjerg, 2003; Valdimarsdottir et al., 1995; Zakowski et al., 1997), functioning as a background stressor

sufficient to increase acute stress reactivity to independent stressors (Gump & Matthews, 1999).

The goal of the following series of experiments is to investigate HPA axis function, psychosocial correlates, and central adiposity in women at familial risk for breast cancer. Stress through its hormonal correlates and possible association with central adiposity may play a crucial role in the etiology of breast cancer in women with family histories of the disease.

2 Theoretical Background

2.1 Breast Cancer: Statistics and Epidemiology [1]

Incidence: Breast cancer is the most frequently diagnosed cancer in women with an estimated 32% of US cancer cases, next to all skin cancers combined. The number of new cases in the United States alone during 2004 is estimated to be 275,380 (215,990 new cases of invasive breast cancer and 59,390 new cases of in situ breast cancer) (American Cancer Society, 2004). Approximately 1 in 7 women in the United States will develop breast cancer over her lifetime. Breast cancer incidence rates have continuously increased since 1980 in women age 50 and older, albeit with a slower rate in the 1990s. *Mortality:* Even though mortality rates have decreased by 2.3% per year during the last decade, breast cancer is still the second leading cause of cancer deaths in women (after lung cancer) with an estimated 40,110 deaths for 2004 in the United States. Breast cancer accounts for 15% of all estimated US cancer deaths. The estimated 5-year relative survival rate from breast cancer is 87%. Survival rates markedly increased for breast cancer with a 75% rate between 1974-1976, 78% between 1983-1985, and 87% between 1992-1999. *Disparities:* There are ethnic and racial disparities in breast cancer incidence and mortality. Breast cancer incidence is highest among white women (140.8 per 100,000[2]), followed by African American (121.7 per 100,000), Asian American and Pacific Islander (97.2 per 100,000), American Indian and Alaska Native (58.0 per 100,000). The incidence rate for Hispanic Latinos, which is not mutually exclusive from the rest, is 89.8 per 100,000.

[1] Breast Cancer Statistics in this section of the introduction are taken from the American Cancer Society, Cancer Facts and Figures 2004.
[2] All rates are per 100,000, age-adjusted to the 2000 US standard population.

Breast cancer death rates are highest among African-American women (35.9 per 100,000), followed by white women (27.2 per 100,000), American Indian and Alaska Native (14.9 per 100,000), and Asian American and Pacific Islander (12.5 per 100,000). The death rate for Hispanic Latinos, which is not mutually exclusive from the rest, is 17.9 per 100,000. Internationally, the United States rank on number 12 for breast cancer death rates out of 45 countries (Germany ranks on number 8). The highest breast cancer death rates are found in Denmark and the Netherlands (rank 1 and 2, respectively), and the lowest breast cancer death rates are found in Japan and China (rank 44 and 45, respectively).

Epidemiology: As indicated by incidence and mortality rates, breast cancer poses a serious public health problem, and efforts to understand the etiology of the disease are essential. Numerous studies have been dedicated to the identification of factors that contribute to the development of breast cancer (Dumitrescu & Cotarla, 2005) (for review)[3]. These efforts have revealed a number of risk factors for breast cancer which can be categorized as genetic and nongenetic, and further subdivided into hormonal and nonhormonal for the nongenetic risk factors (Martin & Weber, 2000). Consistent with a genetic influence on breast cancer risk, it is well understood that one of the most important risk factors for developing breast cancer is a family history of the disease (Collaborative Group, 2001). Genetic susceptibility of breast cancer is widely being associated solely with the known high-penetrance mutations of the BRCA1 and BRCA2 genes. Other genetic factors have been discovered including other high-penetrance genes (p53, ATM, NBS1, LKB1), low-penetrance genes such as cytochrome P450 genes

[3] A more comprehensive review of the well established risk factors for breast cancer can be found in Dumitrescu et al.'s article; here, I present a very brief summary of the epidemiology of breast cancer as part of the introductory chapter

(CYP1A1, CYP2D6, CYP19), glutathione S-transferase family (GSTM1, GSTP1), alcohol and one-carbon metabolism genes (ADH1C and MTHFR), DNA repair genes (XRCC1, XRCC3, ERCC4/XPF), and genes encoding cell signaling molecules (PR, ER, TNFalpha or HSP70) (Dumitrescu et al., 2005) (for review). However, effects of all known gene mutations suspected to influence breast cancer risk in women at familial risk for the disease have been estimated to account for only 20-25% (Easton, 1999). Furthermore, population based studies show that after accounting for the contribution of mutations on BRCA1 and BRCA2, family history is still associated with a significant increase in lifetime risk of breast cancer (Claus, Schildkraut, Iversen, Jr., Berry, & Parmigiani, 1998; Kaufman & Struewing, 1999). Familial breast cancer risk will be discussed in more detail later in this chapter (see section 2.2.2) as it is one of the main elements of the proposed familial breast cancer model.

Consistent with a nongenetic hormonal influence on breast cancer risk, several hormonal risk factors have been thoroughly studied (Bernstein & Ross, 1993; Dumitrescu et al., 2005; Martin et al., 2000). Estrogen exposure has been repeatedly shown to be associated with the risk of developing breast cancer (Begg et al., 1987; Pike et al., 1979), whereas reducing exposure is thought to be protective (Hulka, 1997). In accordance with these associations, factors that increase the number of menstrual cycles, such as early age at menarche, nulliparity (Kampert, Whittemore, & Paffenbarger, Jr., 1988; White, 1987), and late onset of menopause (Trichopoulos, MacMahon, & Cole, 1972) are associated with an increased likelihood of developing breast cancer, whereas factors that decrease the number of menstrual cycles appear to be protective, which can be achieved by moderate levels of exercise (Bernstein, Henderson, Hanisch, Sullivan-Halley, & Ross,

1994) and a longer lactation period (Yuan, Yu, Ross, Gao, & Henderson, 1988). Further hormonal factors associated with an increased risk of developing breast cancer, albeit with some inconsistencies in the literature are the use of postmenopausal hormone replacement therapy (Agarwal & Judd, 1999; Steinberg et al., 1991), and increasing maternal age at first live birth (MacMahon et al., 1970). Finally, anthropometric characteristics such as height and obesity, which is a major source of estrogen through conversion of androstenedione to estrone by adipose tissue are related to increased breast cancer risk in postmenopausal but not premenopausal women (Paffenbarger, Jr., Kampert, & Chang, 1980; Pujol, Galtier-Dereure, & Bringer, 1997). More recently, fat accumulation around the waist (central fat distribution or central adiposity) has been shown to be a risk factor for breast cancer in premenopausal and postmenopausal women (Connolly et al., 2002) (for review), and waist measurements with adjustment for general obesity in premenopausal women (Harvie, Hooper, & Howell, 2003b). As central adiposity is another main element of the proposed familial breast cancer model, it will be discussed in detail later in this chapter (see section 2.2.5), as well as in the respective chapters on central adiposity in women at familial risk for breast cancer (see chapter 6 and 7).

The counterpart to hormonal risk factors is nonhormonal risk factors. However, some of these nonhormonal risk factors may be indirectly linked to the modulation of estrogen exposure. One such factor is exposure to ionizing radiation as women who received mantle radiation for Hodgin's lymphoma have experienced (Bhatia et al., 2003; Wolden, Lamborn, Cleary, Tate, & Donaldson, 1998) or survivors of the atomic bomb blasts in Japan (Land, 1995). Another risk factor in this category is alcohol consumption,

which amount and duration has been linked to increased breast cancer risk (Bowlin et al., 1997; Garfinkel, Boffetta, & Stellman, 1988; Land, 1995), possibly through an increase in exposure to estrogen (Nagata, Kabuto, Takatsuka, & Shimizu, 1997) through cirrhosis and its associated hormonal disruptions (Gavaler, 1995) and the fact that plants used to produce alcoholic beverages contain estrogen-like substances (i.e., phytoestrogens) (Gavaler, 1998). There is evidence that certain dietary factors may also contribute to an increased risk for developing breast cancer, albeit with some controversy due to study bias, discrepant data, and difficulties with assessing dietary-exposure histories. These dietary risk factors for breast cancer include high dietary fat (Boyd et al., 2003; Wynder et al., 1997) and "well-done" meat (Zheng et al., 1998; Zheng et al., 1999). Finally, characteristics of the breast, such as proliferative benign breast pathology and mammographic density increase the risk of developing breast cancer (Cuzick, 2003) (for review).

Little is known about the possible interaction between these factors on breast cancer risk, and it is only speculated that nonhormonal risk factors contribute to breast cancer development in relationship to common variant alleles of a variety of genes. A small number of studies reporting on overlapping samples has indicated an interaction between anthropometric factors and family history of breast cancer on breast cancer risk with a more pronounced increase in the risk of disease associated with a high waist-to-hip ratio among women with a family history of breast cancer (Sellers et al., 1992; Cerhan et al., 2004; Sellers et al., 2002; Olso, Anderson, Cerhan, Follsom, & Sellers, 2000). It is possible, that certain breast cancer risk factors may be associated with a more pronounced increase in breast cancer risk in women at familial risk for the disease, which may

identify differing etiologic pathways in women with and without family histories of breast cancer. According to the current state of research, it appears, that risk factors independently relate to breast cancer risk which allows for the estimation of an individual's risk of developing breast cancer (Cuzick, 2003).

Consecutively, a familial breast cancer model will be outlined, which is, on one hand, based on epidemiological findings indicating central adiposity to be a risk factor for breast cancer, and on the other hand, based on psychoneuroendocrinological studies suggesting an association between central adiposity, HPA axis dysfunction, and greater psychological vulnerability to stress. Both, central adiposity and HPA axis reactivity in addition to self-reported distress, have been reported independently to be increased in women with family histories of breast cancer (Erblich, Bovbjerg, & Valdimarsdottir, 2000; Erblich, Montgomery, Valdimarsdottir, Cloitre, & Bovbjerg, 2003; Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003; Schapira, Kumar, & Lyman, 1993).

2.2 Familial breast cancer model: role of psychological distress, hypothalamus-pituitary-adrenal (HPA) axis function, and central adiposity

2.2.1 Introduction

The higher incidence of breast cancer among women with family histories of the disease can only partly be attributed to known primary susceptibility genes (i.e., BRCA1, and BRCA2). Additional factors must exist. Taking together findings from epidemiological as well as psychoendocrinological studies consistently showing: a)

central adiposity to be a risk factor for breast cancer, b) a higher incidence of central adiposity among women with family histories of breast cancer, c) a relationship between central adiposity, increased HPA axis reactivity resulting in increased levels of the axis' end product cortisol, and greater psychological vulnerability to stress, and d) increased cortisol levels in response to laboratory stress in women with family histories of breast cancer, in addition to self-reported distress, leads to a new model potentially explaining part of the increased family incidence of breast cancer from a psychoneuroendocrinological perspective.

The goal of the following series of experiments is to investigate HPA axis function, psychosocial correlates, and central adiposity in women at familial risk for breast cancer. A higher incidence of HPA axis dysfunction (in the form of HPA axis hyperactivity), psychological distress, and central adiposity in this population may indicate a greater health risk. Below is a brief review of the factors included in the proposed theoretical model (Figure 1). These factors will be addressed in more detail in the respective chapters that are following the introduction (see chapters 3 through 7).

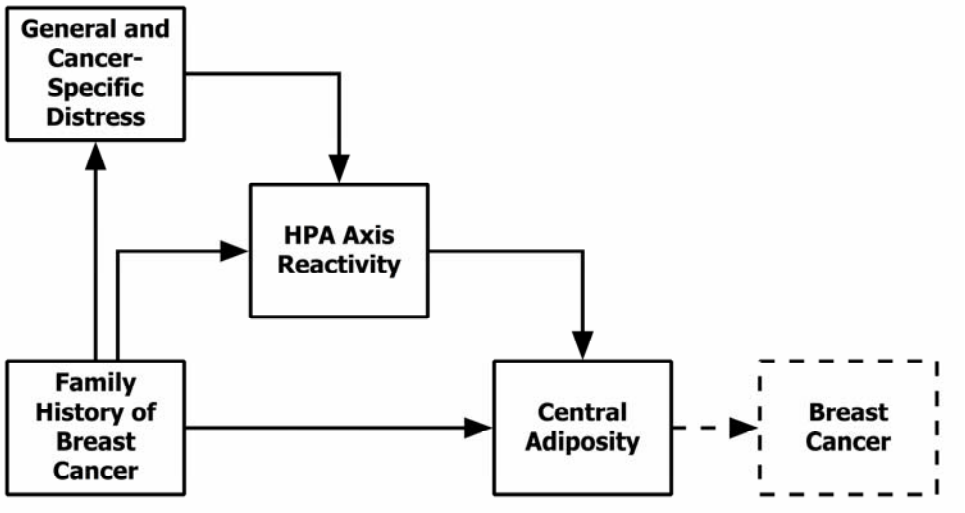

Figure 1. Stress through its hormonal correlates and possible association with central adiposity may play a crucial role in the etiology of breast cancer in women with family histories of this disease.
Note. Development of breast cancer is not assessed in the cross-sectional design of women with and without family histories of breast cancer in the current work

2.2.2 Family history of breast cancer and lifetime risk of developing breast cancer

A long recognized risk factor for breast cancer is a family history of the disease (Pharoah et al., 2002). Having even a single first-degree relative with breast cancer up to doubles a woman's lifetime risk of developing the disease herself (Collaborative Group, 2001). The risks increase further if other characteristics consistent with inherited susceptibility are present in the family, such as multiple affected relatives and younger ages at diagnosis (i.e., before age 50) (Hopper, 2001). Premenopausal women with a family history of breast cancer have particularly high risks of developing breast cancer at a younger age suggesting a stronger contribution of genetics to premenopausal breast cancer (Pharoah, Day, Duffy, Easton, & Ponder, 1997). Overall, the risk of developing

breast cancer is 1.9-3.9 times higher in women with an affected mother or sister (Collaborative Group, 2001).

Over a decade ago, linkage analyses of large kindreds with multiple affected family members over several generations led to the identification of two large genes, BRCA1 located on chromosome 17q21 (Hall et al., 1990) and BRCA2 located on chromosome 13q12-13 (Wooster et al., 1994), both tumor suppressor genes whose primary function is maintaining genomic integrity (caretaker gene) (Deng & Brodie, 2000). Having a germline mutation in either of these genes is associated with an increased risk of breast cancer of at least 10-fold and a lifetime risk of breast cancer somewhere between 40-80% (Fisher, Kirk, Hopper, Godding, & Burgemeister, 2003). In western countries, the proportion of the general population who carry a mutation is thought to be about 1 in 800 to 1 in 500, although it is about 1 in 40 in those of Ashkenazi Jewish descent (Cipollini et al., 2004). In women with a diagnosis of breast cancer, less than 10% of cases appear to have a detectable mutation, and among healthy women with at least one first or second degree relative with breast cancer and/or ovarian cancer at the age of 49 or younger the prevalence ranges from 4.4% (breast cancer in one relative before the age of fifty and no ovarian cancer in any relative) to 16.4% (breast cancer before the age of 50 and ovarian cancer at any age) (Frank et al., 2002; Cipollini et al., 2004). Population based studies have indicated that after accounting for the contribution of mutations on these two known breast cancer susceptibility genes to risk, family history is still associated with a significant increase in lifetime risk of breast cancer (Claus et al., 1998; Kaufman et al., 1999). For breast cancer cases in families with four or more affected relatives, mutations in the BRCA1 and/or BRCA2 genes are thought to be

responsible (Ford, Easton, & Peto, 1995), but overall, the contribution of mutations in BRCA1 and BRCA2 to familial risk has been estimated to be only 15% (Pharoah et al., 2002), and the effects of all known gene mutations suspected to influence breast cancer risk including other high-penetrance genes (p53, ATM, NBS1, LKB1), low-penetrance genes such as cytochrome P450 genes (CYP1A1, CYP2D6, CYP19), glutathione S-transferase family (GSTM1, GSTP1), alcohol and one-carbon metabolism genes (ADH1C and MTHFR), DNA repair genes (XRCC1, XRCC3, ERCC4/XPF), and genes encoding cell signaling molecules (PR, ER, TNFalpha or HSP70) (Dumitrescu et al., 2005) (for review) have been estimated to account for 20-25% of familial risk (Easton, 1999). Hence, other risk factors for explaining the increased susceptibility among women with family histories of breast cancer must exist.

2.2.3 Psychological distress in women at familial risk for breast cancer

Having a family history of breast cancer is considered to be a source of stress with aspects of uncontrollability and helplessness due to several reasons: a) individuals face the risk of developing the disease themselves since having a family history of breast cancer is one of the strongest predictors for development, b) individuals also face the possible loss of a loved one, and c) caring for a family member with breast cancer is thought to be a challenge and stress. According to cognitive stress theory (Lazarus & Folkman, 1984), an evaluation of a threat to one's life is a major factor leading to stress.

Based on this model, women with family histories of breast cancer are bound to experience stress, as the most robust finding in the psychological literature on healthy women with family histories of breast cancer are higher perceived breast cancer risk

estimates, as recently summarized by a meta-analytic review of twelve studies incorporating a total of 70,660 participants (Katapodi, Lee, Facione, & Dodd, 2004). Given this pattern of findings, it is not surprising that a growing body of evidence suggests that women with family histories of breast cancer experience chronic psychological distress, as operationalized by higher levels of general distress and negative affect (Baider, Ever-Hadani, & Kaplan De-Nour, 1999; Valdimarsdottir et al., 1995), as well as cancer-specific distress, such as intrusions about the disease (Baider et al., 1999; Valdimarsdottir et al., 1995; Zakowski et al., 1997). They report higher levels of both intrusive thoughts and general distress than a comparison group of women at population risk for breast cancer even long after the diagnosis of breast cancer in their first degree relatives (mean 14.4 years) (Erblich et al., 2003), and after they have recently learned that their mammogram results are normal (Zakowski et al., 1997). However, while in some studies approximately one out of four women had levels of distress that would potentially benefit from a psychological intervention (Kash, Holland, Halper, & Miller, 1992), and approximately one out of three reported that their daily lives were affected by worries about breast cancer (Lerman et al., 1993), a few refute the hypothesis that women with a family history of breast cancer are at greater psychological risk (Butow et al., 2004; Coyne, Benazon, Gaba, Calzone, & Weber, 2000).

2.2.4 HPA axis dysfunction in women at familial risk for breast cancer

The results on the type and time course of psychological distress in women at familial risk for breast cancer support feelings of uncontrollability and helplessness among women with family histories of breast cancer, which is the type of stress that is

found to lead to a dysregulation of the HPA axis (Dickerson & Kemeny, 2004; Peters et al., 1998; Peters et al., 1999) resulting in increased levels of the axis' end product cortisol. Cortisol is a steroid hormone that in humans is the major circulating hormone of the adrenal gland. Cortisol is classified as a glucocorticoid and is synthesized and secreted by the adrenal cortex in response to adrenocorticotropic hormone (ACTH) released from the pituitary, which secretion is stimulated by corticotropin releasing hormone (CRH) released by the hypothalamus . Cortisol is the major regulator of ACTH production in the pituitary gland and CRH production in the hypothalamus; it acts by negative feedback inhibition, i.e., a rise in the level of cortisol in the blood inhibits ACTH secretion by the pituitary and CRH secretion by the hypothalamus (Columbia Encyclopedia, 2004).

Research results lend support for the assumption that first-degree relatives of breast cancer patients might have perturbations of the regulation of the HPA axis. For example, in response to a standardized laboratory stressor, healthy women with family histories of breast cancer showed elevated salivary cortisol responses compared to women without family histories of the disease (Gold et al., 2003). In another study, morning plasma cortisol levels were increased in daughters with mothers diagnosed with breast cancer one year prior to the beginning of the study, with highest levels among daughters who's mothers were diagnosed with recurrent breast cancer (Cohen, 2002). Other psychobiological indices known to be linked to the secretory pattern of the HPA axis (Elenkov & Chrousos, 2002; Miller & O'Callaghan, 2002; Sapolsky, Romero, & Munck, 2000) (for review) have been found to differ in women at familial risk for breast cancer: In response to a standardized laboratory stressor, women at familial risk for breast

cancer showed larger increases in distress, heart rate, natural killer cell activity, and natural killer cell numbers (Valdimarsdottir et al., 2002).

Consistent with heightened neuroendocrine reactivity to experimental stressors, in response to stressors in ordinary life (work stress), women at familial risk of breast cancer exhibited increased urinary epinephrine excretion (a catecholamine together with norepinephrine, secreted by the medulla of the adrenal gland) during work, but not during home or sleep. According to the view of a joint effort of the HPA axis and the sympathoadrenal medullary system (SAM) to mediate the stress response with catecholamines acting in concert with cortisol (Nicolaidis, 2002; Sapolsky et al., 2000), here, HPA responses using the naturalistic approach of collecting urinary cortisol during work as the stressful period of the day, home, and sleep will be investigated (see chapter 3).

2.2.5 Central adiposity as a risk factor for breast cancer

The relationship between general obesity and breast cancer risk has long been recognized (de Waard, Baanders-Vanhalewijn, & Huizinga, 1964) but more recently, studies have suggested that central rather than general obesity may be linked to the development of breast cancer (Bruning et al., 1992; Connolly et al., 2002; Stoll, 2002), and specifically central adiposity adjusted for general obesity in premenopausal women (Harvie, Hooper, & Howell, 2003a). Central adiposity is characterized by excess fat around the waist and is also referred to as central fat, or abdominal fat[4]. Abdominal fat is composed of subcutaneous abdominal fat and intra-abdominal visceral fat (Matsuzawa et al., 1995). Visceral fat is morphologically different from subcutaneous and peripheral fat.

[4] these terms will be used interchangeable throughout this work

It has greater blood flow and up to four times more glucocorticoid receptors than peripheral fat making it especially sensitive to the fat-accumulating effects of circulating cortisol (Pedersen, Jonler, & Richelsen, 1994). Visceral fat is considered an endocrine organ due to its distinct morphology and ability to convert inactive cortisone into active cortisol and the reverse by the enzymes 11beta-hydroxysteroid dehydrogenase type 1 and 2, respectively (Jazet, Pijl, & Meinders, 2003; Cinti, 2001; Trayhurn & Beattie, 2001; Faloia, Camilloni, Giacchetti, & Mantero, 2000).

Ideally, abdominal fat is assessed with direct measurements using imaging techniques allowing a clear distinction between the two fat compartments. However, most large epidemiological studies have used surrogate measures of abdominal fat, such as waist circumference and waist-to-hip ratio (WHR). WHR measures body fat distribution by capturing the relative accumulation of abdominal compared with gluteal fat (Despres, Lemieux, & Prud'homme, 2001). Waist circumference alone is a correlate of the amounts of visceral and subcutaneous fat together (Seidell et al., 1987). The use of waist circumference in combination with BMI is a better predictor of visceral fat alone with the addition of waist circumference to BMI explaining an additional 16% of the variation in visceral fat but not abdominal subcutaneous fat (Janssen, Heymsfield, Allison, Kotler, & Ross, 2002). Due to the different morphology of visceral fat, it is not surprising that waist circumference predicts health risk beyond that predicted by BMI alone (Janssen et al., 2002).

A recent meta-analysis of 19 studies on WHR and risk of breast cancer found a clear association for premenopausal women and postmenopausal women (Connolly et al., 2002). Despite the large amount of methodological variation among studies, summary

risk estimates were above one for all analyses in case-control and cohort studies when divided by menopausal status and for each study design in total, as well as for all premenopausal women, all postmenopausal women, and all studies combined. Risk estimates were of greater magnitude for premenopausal than for postmenopausal women. Because waist measurements and WHR are highly correlated with general obesity, a factor that has strong positive associations with postmenopausal breast cancer and an inverse correlation with premenopausal breast cancer, a more recent systematic review of central obesity as a risk factor for breast cancer aimed to evaluate whether adjustment for body mass index (BMI) modified the relationship between waist circumference or WHR and breast cancer risk in pre- or postmenopausal women (Harvie et al., 2003b).

Consistent with Connolly and colleagues' meta-analysis (Connolly et al., 2002), the results without adjustment for BMI indicated lower risks of breast cancer in postmenopausal women with small waist circumference and small WHR, however, in premenopausal women no such relations were found. Interestingly, adjusting for BMI attenuated the relationship between waist and WHR and breast cancer risk in postmenopausal women, but produced such a relationship in premenopausal women. Hence, it appears that the relationships between waist and WHR with breast cancer risk in postmenopausal women result from the high correlation between waist and WHR with BMI. In premenopausal women, however, central and not general obesity may be specifically associated with breast cancer risk. It is important to note with regard to inconsistencies between the two reports, that the systematic review used much stricter inclusion criteria identifying only eight studies for inclusion in the final analysis, whereas the meta-analysis included 19 studies. One such exclusion for the systematic review was

waist measurements after commencing breast cancer treatment with the reasoning that chemotherapy and endocrine therapy have been associated with central fat gains irrespective of increases or decreases in body weight (Cheney, Mahloch, & Freeny, 1997).

2.2.6 Central adiposity in women at familial risk for breast cancer

The prevalence of central adiposity as a well-established risk factor for breast cancer in women at familial risk for the disease has only been addressed in one study conducted over a decade ago (Schapira et al., 1993). The authors reported significantly larger WHRs in 56 healthy women with family histories of breast cancer compared to 56 healthy women without such histories controlling for overall adiposity. A replication of these findings is of high importance, as it would suggest the possibility of interventional strategies to reduce the risk of breast cancer in families with a history of the disease by reducing abdominal fat. Studies have shown that exercise programs are associated with significant reductions in total adiposity and abdominal fat, even after controlling for reductions in waist circumference and BMI, respectively, indicating reductions in visceral fat, the fat compartment that is thought to be responsible for increased health risks due to its endocrine characteristics (Janssen et al., 2004). To confirm the previous result of higher WHR in women with family histories of breast cancer, it is important to consider potentially confounding characteristics that may be related to central obesity and breast cancer risk, such as menopausal status or parity (see chapter 6).

2.2.7 Central adiposity and HPA axis dysfunction

A number of investigations have provided support for the idea of a co-occurrence of hypercortisolism and central fat (Bjorntorp, 2001; Dallman et al., 2004) (for review). Various mechanisms have been studied to link cortisol and central adiposity. While some researchers have focused on HPA axis reactivity in the presence of central adiposity with results suggesting a hypersensitive HPA axis and a blunted feedback control by central glucocorticoid receptors (Bjorntorp, 2001; Kopelman et al., 1988; Ljung, Andersson, Bengtsson, Bjorntorp, & Marin, 1996; Marin et al., 1992; Pasquali et al., 1993; Rosmond, Dallman, & Bjorntorp, 1998; Rosmond, Holm, & Bjorntorp, 2000), which is also true for lean women with a central distribution of fat (Epel et al., 2000), other researcher point out the important role of glucocorticoids to regulate adipose tissue (Bujalska, Kumar, & Stewart, 1997; Dallman et al., 2004; Gaillard, Wabitsch, Pipy, & Negrel, 1991; Hauner, Schmid, & Pfeiffer, 1987). Animal studies have shown that chronic physical and psychological stress exposure is characterized by visceral fat deposition, insulin resistance, hyperinsulinemia, impaired glucose tolerance, dislipidemia, and premature artherosclerosis (all characteristics of the diagnostic category of the metabolic syndrome) (Dallman, Akana, Bhatnagar, Bell, & Strack, 2000) (for review). Dallman and colleagues state that glucocorticoid receptor signaling throughout the circadian cycle in response to chronic stress increases gluconeogenesis, insulin secretion, obesity, muscle wasting, bone loss, and immune suppression in rats. They conclude that chronic mild stress, through its effect on HPA axis reactivity throughout the day, facilitates the development of the metabolic syndrome. The activity through which cortisol leads to accumulation of central fat involves several processes: on one hand, cortisol increases sweet, high-fat food

ingestion after a stressor (Epel, Lapidus, McEwen, & Brownell, 2001), and on the other hand cortisol inhibits the lipid mobilizing system in adipocytes, which is mediated by the higher density of glucocorticoid receptors in central fat compared to other fat depots resulting in a glucocorticoid-mediated redistribution of stored calories into abdominal fat (Bjorntorp, 2001). Abdominal fat sends an unidentified signal to the brain to reduce the overall level of activity of the chronic stress response network, which explains the use of the term "comfort food" (Dallman et al., 2004). Also, intra-abdominal fat has the unique ability to produce cortisol from its inactive precursor cortisone and also to synthesize leptin which plays a central role in controlling body weight and regulating fat stores (Pantanetti et al., 2004). Hence, intra-abdominal fat and increased glucocorticoids appear to co-occur in a vicious cycle. Last, evidence exists for common polygenic but not major gene determinants underlying cortisol and abdominal fat covariation accounting for 16% to 20% of the phenotypic variances (Feitosa et al., 2002).

2.2.8 Overview of present work

In sum, as indicated by incidence and mortality rates, breast cancer poses a serious public health problem. It is a disease which is associated with fear and psychological distress, particularly for women who due to a family history of the disease are at increased risk of developing breast cancer. Familial risk for the disease can only partly be attributed to known primary susceptibility genes (i.e., BRCA1 and BRCA2). Hence, efforts to understand the etiology of the disease, particularly for women at yet partly unexplained increased risk are essential. Given the pattern of findings that women with a family history of breast cancer indicate higher levels of psychological distress, as

well as increased physiological reactivity to acute stressors, and that these responses, in a separate literature, have been associated with central body fat localization, a condition that has been repeatedly shown to be a risk factor for breast cancer with a more pronounced increase in the risk of breast cancer associated with a high waist-to-hip ratio among women with a family history of breast cancer (Sellers et al., 1992) leads to a new psychobiological model suggesting etiologic differences between familial breast cancer and the sporadic form. In sum, the present work aims to present empirical data from a naturalistic study design to investigate a new psychobiological model by which stress through its hormonal correlates and possible association with central adiposity may play a crucial role in the etiology of breast cancer in women at familial risk of the disease.

Consecutively, a series of papers will be presented. First, I will investigate cortisol responses to daily stress in working women at familial risk for breast cancer by using a naturalistic design, where the work period of the day is operationalized as the stressful time of the day, compared to home, and sleep (see chapter 3). Second, I will present analyses in healthy population-risk women only to investigate whether breast cancer specific distress is related to cortisol responses to daily life stressors even in a group of women who do not have a family history of breast cancer (see chapter 4). Third, I will include the familial risk group again, but I will limit the analysis to premenopausal women and further subdivide women with first-degree relatives diagnosed at postmenopausal age (low familial risk, LoFR) vs. at premenopausal age (high familial risk, HiFR), as highest risk estimates exist for premenopausal women with first-degree relatives diagnosed at premenopausal age (Collaborative Group, 2001). In this sample, I will explore the possibility that breast cancer specific distress mediates higher levels of

cortisol reactivity in women at familial risk for the disease (see chapter 5). Fourth, I will present findings on levels of abdominal fat controlling for overall adiposity in premenopausal women at high, low, and no familial risk for breast cancer, as this measure of central fat, specifically, is associated with breast cancer risk in premenopausal women according to a recent review (Harvie et al., 2003b) (see chapter 6). Fifth, exploratory analysis will investigate associations between cortisol reactivity and abdominal fat in premenopausal women at different familial risks (no, low, high) for breast cancer (see chapter 7). The final chapter consists of a general discussion of the results and the empirical support for the familial breast cancer model proposed in this work (see chapter 8).

Heightened cortisol responses to daily stress in working women at familial risk for breast cancer[1]

Abstract

Consistent with animal models and experimental studies with humans facing other "background" stressors, women at familial risk for breast cancer have been reported to have stronger cortisol responses to laboratory stressors. To explore the relevance of these findings to daily life, we compared work-stress cortisol responses in women with ≥1 first-degree relative with breast cancer (FH+, n=74) to women without this risk factor (FH-, n=141). Repeated measures ANOVA revealed a Group by Time interaction ($p \leq 0.05$) with FH+ women having higher ($p \leq 0.05$) urinary cortisol levels than FH- during work, but not at home or during sleep. They also had a higher percentage increase between nadir cortisol levels and work levels. These results provide evidence that the heightened cortisol responses of FH+ women also apply to daily life stressors, and suggest the need for additional research to explore the possibility that accentuated hypothalamic-pituitary-axis responses to such stressors may increase health risk for these women.

KEYWORDS: Breast cancer – Familial risk – Cortisol – Work stress

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Introduction

The potential implications of stress-induced systematic alterations of cortisol for a variety of human illnesses have long been noted (Chrousos & Gold, 1992; Chrousos & Gold, 1998). Cortisol is secreted by the adrenal gland in response to release of adrenocorticotropin hormone (ACTH) by the pituitary, which in turn is stimulated by release of corticotrophin-releasing hormone (CRH) by the hypothalamus. Activation of the hypothalamus-pituitary-adrenal (HPA) axis follows disruption of homeostasis and constitutes an essential element of mediating the stress response (Miller & O'Callaghan, 2002). Under basal conditions, cortisol follows a distinct circadian rhythm with highest levels usually shortly after awakening, sharp decreases during the first few hours in the morning, more gradual decreases thereafter, and increased production again during the night (Posener, Schildkraut, Samson, & Schatzberg, 1996). Physical and medical implications of cumulative exposure to increased cortisol levels are potentially substantial, because cortisol binds to glucocorticoid receptors, which are present in almost every tissue and organ in the body. Cortisol mediates many metabolic processes ranging from induction of liver enzymes involved in energy metabolism to regulating the trafficking of immune cells and cytokine production (McEwen & Seeman, 1999).

We have been investigating stress associated with having a family history of breast cancer in first degree relatives, which increases a woman's lifetime risk of developing the disease by up to four times (Evans & Lalloo, 2002). Even though detection at earliest stages promises a 95% 5-year survival rate, breast cancer is the second leading cause of cancer mortality next to lung cancer in American women (ACS 2004). While breast cancer was surrounded by secrecy until the 1980s, an increase in

consumer advocacy and media coverage has brought the condition to the attention of the public, as well as the adverse side effects of treatments, including disfiguring surgery, radiation and chemotherapy (Baum, 2000).

Accordingly, breast cancer is one of the most feared of all diseases as identified by a national survey of 1045 women (Spittle & Morgan, 1999). The threat of breast cancer is even more pronounced for women with family histories of the disease as it increases a woman's risk of developing the disease (Rees, Fry, & Cull, 2001). Women with family histories are also likely to have witnessed first hand the adverse physical and emotional consequences of diagnosis and treatments; they have confronted an immediate challenge to established family dynamics and may have lost a loved one (Welch, Wadsworth, & Compas, 1996; Zahlis, 2001). Reminders of breast cancer have been found to bring back strong feelings in women with family histories of breast cancer (i.e., Erblich, Montgomery, Valdimarsdottir, Cloitre, & Bovbjerg, 2003). Furthermore, with regard to their perceptions of their own risk of developing breast cancer, survey studies have consistently found that women with positive family histories of breast cancer overestimate their risk (Erblich et al., 2003; Evans, Burnell, Hopwood, & Howell, 1993; Helzlsouer, Ford, Hayward, Midzenski, & Perry, 1994; Mouchawar, Byers, Cutter, Dignan, & Michael, 1999). Considerable empirical evidence that a family history of breast cancer is a major life stressor for many women stems from studies within this population reporting adverse psychological (Bovbjerg & Valdimarsdottir, 2001), cognitive (i.e., Erblich et al., 2003), and biological processes (Cohen, 2002; Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003; Valdimarsdottir et al., 2002; James, Berge-Landry, Valdimarsdottir, Montgomery, & Bovbjerg, 2004).

Until now, very little attention has been paid to cortisol under daily life conditions in women at familial risk of breast cancer compared to those at population risk. The goal of the present study was to evaluate cortisol responses in naturalistic settings (i.e., work, home, and sleep) in daily life outside the laboratory. We were particularly interested in responses to work stress, since prior research has indicated that the workplace is the site of the strongest stress responses during the course of an ordinary day (i.e., James & Brown, 1997). We hypothesized that women with family histories of breast cancer would have higher cortisol excretion rates with work stress compared to women without family histories of the disease, as well as a greater percentage increase from nadir (i.e., sleep) to work levels.

Support for an increased work stress response in cortisol stems from previous experimental data showing stronger cortisol responses to classic experimental stressors in a laboratory study of women with and without family histories of breast cancer (Gold et al., 2003). This hypothesis is also consistent with a large experimental literature documenting increased acute stress responsivity in animals under various chronic stress conditions (i.e., Bhatnagar & Dallman, 1998), as well as experimental studies with humans confronting different chronic stressors such as long-term unemployment, burnout, and work overload (i.e., Ockenfels et al., 1995; Cacioppo et al., 2000; Wust, Federenko, Hellhammer, & Kirschbaum, 2000; Ockenfels et al., 1995; De Vente, Olff, Van Amsterdam, Kamphuis, & Emmelkamp, 2003; Schlotz, Hellhammer, Schulz, & Stone, 2004). Gump and Matthews (Gump & Matthews, 1999) introduced the supraordinate term “background stressor” to describe enduring stressors with varying duration of stressor exposure.

The possibility that women facing the background stress of being at familial risk for breast cancer may have increased responsivity to stresses outside the laboratory is also supported by our recent naturalistic study indicating increased reactivity of the other classic stress response system, the sympathetic adrenomedullar system (SAM) to an ordinary life stressor (work). The women at familial risk for breast cancer had higher rates of epinephrine excretion while at work and a greater percentage increase in epinephrine and norepinephrine from sleep to work compared to women at population risk (James et al., 2004).

Alternatively, it is also possible that the background stress of having a family history of breast cancer may affect the circadian rhythm of cortisol secretion. Considerable research in animals and humans has shown that the usually very persistent circadian rhythm of cortisol can be modulated by stressful experiences of varying intensity. It has been suggested that persistent mild stress results in elevated trough cortisol levels (Brennan, Ottenweller, Seifu, Zhu, & Servatius, 2000; Cella, Van Cauter, & Schoeller, 1995; Linkowski et al., 1987; Linkowski, 2003; Van Cauter, Leproult, & Kupfer, 1996; Dallman, Akana, Bhatnagar, Bell, & Strack, 2000). One of the major theories to explain increased levels of the nocturnal cortisol nadir with exposure to chronic stress is a vicious cycle of cumulative exposure to cortisol. It has been speculated that higher cortisol levels can cause degenerative processes in the central nervous system, resulting in the impairment of slow feedback mechanisms, which then leads to the inability of the HPA axis to regulate cortisol release (Lupien & Lepage, 2001; Sapolsky, Krey, & McEwen, 1986; Dallman et al., 2000; Sheline, 2003). In support of this theory, Van Cauter et al. (Van Cauter et al., 1996) found elevations in mean cortisol levels, levels

of the nocturnal nadir, and the morning acrophase with increased age, a surrogate measure of cumulative exposure to stress.

Based on this literature, in addition to our hypothesis of increased reactivity to work stress in women with family histories of breast cancer, we also explored the possibility that these women would have higher trough cortisol levels (cortisol nadir defined as the lowest value on either home or sleep) compared to women without family histories.

Method

Design and Subjects

In order to compare the effects of daily stress on cortisol levels between employed women who had or did not have first-degree family histories of breast cancer, a "natural" experimental design was employed in which their workday was divided into three different microenvironments: work, home and sleep, where work was operationally defined as the stressor condition, based on earlier studies indicating that it is the work place that consistently elicits the strongest stress responses of the day (i.e., Brown & James, 2000; James et al., 1997; Kario, James, Marion, Ahmed, & Pickering, 2002). Urinary cortisol excretion rates of the family history groups were compared across these contrasting daily conditions, as previously described (James et al., 2004).

The subjects of the study were a sample of 215 women who were employed at three major medical centers in New York City. They were recruited through advertisement and had agreed to participate in a study of women with different family histories of breast cancer. The response rate of those women who responded to the ads

was above 90%. The following exclusion criteria for participation in the study were applied: 1) not English speaking, 2) a history of HIV, cancer or abnormal breast exams (including abnormal breast biopsy or abnormal mammogram), 3) medication use other than birth control pills, and 4) participation in any other research study that could potentially affect our study variables. The study was conducted under institutional review board approval and all participants signed informed consent. Based on their self-reported family histories of cancer women were classified into having at least one first-degree relative with breast cancer (FH+) (n=74), or not having a first-degree relative with breast cancer (FH-) (n=141). For this study, only women with complete data on all three cortisol measurements were included in the analyses. It should be noted that a portion of this sample was reported on in James et al.'s study (James et al., 2004) on SAM responses to daily stressors in women with and without family histories of breast cancer and this study includes an additional 61 women.

Procedures

On the morning of the study day, participants met with trained research personnel, completed informed consent, baseline questionnaires including demographic data and medical history, and anthropometric measurements were taken at this time. Participants were provided with a urine collection bottle for the work period at one of the three medical centers where all hospital workers were recruited from and all procedures were explained to them. They were contacted again the same day and two more urine collection bottles were provided to them for the home period and the sleep period. These specimens were returned to the research laboratory the next morning.

The specific urine collection procedures were based on those described by James et al. (James, Schlussel, & Pickering, 1993). Briefly, the first urine specimen at work was not collected but indicated the beginning of the work urine collection period for the study. The participants then collected their urine in the provided 3-liter polyethylene bottle with preservative (0.5 g of sodium metabisulphite) across a block of time (i.e., four hours). This preservative, widely used for this purpose has no known affect on the cortisol assay employed here (Cohen, de Moor, Devine, Baum, & Amato, 2001; Doering et al., 2000; Glover & Poland, 2002). The time of the first and last sample was noted. Identical procedures were followed for the home and sleep sampling periods resulting in samples for the three distinct daily environments defined as work (approximately 11AM to 3PM), home (approximately 6PM to 10PM), and sleep (approximately 10PM to 6AM). The total volume of each sample and the length of time of the collection were recorded for each collection period, so that cortisol values could be corrected by volume and expressed as rate of excretion (µg/24 hours). Potential collection confounding was addressed by preliminary analyses comparing urinary volumes between the two groups for each of the three time blocks, which revealed no significant differences. We chose not to assess creatinine because a considerable literature indicates that urinary creatinine levels are highly variable both within and between individuals and thus cannot provide a reliable indicant of the adequacy of urinary sampling, or serve as a valid referent for other urinary metabolites (i.e., Vestergaard and Leverett, 1958; James et al, 1988). In preparation for the cortisol assay, a 5 ml aliquot was taken from each sample and stored at –60 ^{0}C for future batch assay. Concentrations of cortisol were determined using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA) with a

sensitivity of 0.2 µg/dL and inter- and intra-assay coefficients of variance below 7% for all analyses.

Statistical analysis

For outliers on the dependent variable of cortisol an algorithm of four standard deviations above the mean for each study group (FH+ and FH-) was established. The total listwise deletions due to extreme values on any of the three cortisol measures were six (two in the FH-, and four in the FH+ group). Following the tradition in urinary cortisol literature (i.e., Doering et al., 2000; Cohen et al., 2001) no further manipulations of the data were applied. As ANOVA is robust with regard to non-normality, transformation of such data is not necessary (Neter and Wasserman, 1974; Kupper & Mueller, 1987; Tabachnick & Fidell, 2001). Homogeneity of error variances were adequate as Levene's test did not reveal any significant effects for the dependent variables (work cortisol, home cortisol, sleep cortisol). We determined the cortisol nadir for each participant, the lowest level of the day (home or sleep period). We then computed percent increase from this cortisol nadir to work using the following formula: ((work-nadir)/nadir) x 100. To investigate possible confounding variables (associated with risk factor and outcome), Pearson correlations were computed between the cortisol levels (work, home, sleep, percent increase from the cortisol nadir to work) and continuous variables found to be significantly different between the groups (see below). For group differences on categorical variables, each categorical variable was introduced as a fixed factor in separate repeated measures ANOVAs with daily microenvironment (work, home, sleep) as the repeating factor. Repeated measures ANOVAs with family

history group (FH-, FH+) as a fixed factor and daily microenvironment (work, home and sleep) as a repeating factor were computed to evaluate the daily variation in urinary cortisol excretion. Follow-up one-way ANOVAs were used to evaluate group differences at each time point as well as for percent increase from the cortisol nadir to work.

Results

Table 1 shows selected socio-demographic characteristics of the sample. Overall, the sample was quite diverse: 56.8% of the women were white, 61.4% of the women were single, 70.6% held a college degree, and 93.2% were full-time employed at one of the three medical centers at the time of the study. The remaining 6.8% of women were hospital workers in less than full-time employment situation. No demographic differences were observed between the groups, except for race. As shown in Table 2 group differences in medical history/health-related variables were found only for drinking alcohol on assessment day, and smoking on assessment day.

Table 1: Socio-demographic characteristics of the study sample.[a]

Socio-demographic characteristics	FH- n=141 [b]	FH+ n=74	Statistical test value	p-value
Mean age	37.2 ± 9.2	37.8 ±9.1	F(1,208)=0.231	p≤0.65
Ethnicity -Hispanic	13.1%	8.7%	χ^2=0.880, df=1	p≤0.35
Race -White -Black -Other	53.3% 35.8% 10.9%	64.8% 18.8% 17.4%	χ^2=6.709, df=2	p≤0.04*
Marital Status -currently married	34.8%	45.9%	χ^2=2.566, df=1	p≤0.11
Education -some college or less -College degree -Graduate degree	33.1% 33.1% 33.8%	22.5% 39.4% 38.0%	χ^2=2.230, df=2	p≤0.28
Employment -full time	94.1%	91.4%	χ^2=0.528, df=1	p≤0.47

[a] Mean ± standard deviation or percent (%) of total. FH- Women without a first-degree relative with breast cancer; FH+ Women with at least one first-degree relative with breast cancer.
[b] Number of subjects varies slightly in both groups across variables due to missing values for some factors.
* Significant difference.

Table 2: Medical history variables of the study sample [a]

Medical History	FH- n=141 [b]	FH+ n=74	Statistical test value	p-value
BMI	26.7 ± 6.9	25.3 ± 5.1	F(1,196)=1.972	p≤0.16
Pre-menopausal	79.4%	73.0%	χ^2=1.151, df=1	p≤0.28
Children (≥1)	43.7%	41.3%	χ^2=0.109, df=1	p≤0.74
Birth control pills ever	67.9%	73.9%	χ^2=0.793, df=1	p≤0.37
HRT ever	3.0%	4.3%	χ^2=0.245, df=1	p≤0.62
Cortisone regularly	8.9%	4.3%	χ^2=1.440, df=1	p≤0.23
Smoked on assessment day	4.2%	15.4%	χ^2=6.921, df=1	p≤0.01*
Drank alcohol on assessment day	5.3%	0%	χ^2=3.737, df=1	p≤0.05*
Drank caffeine on assessment day	58.0%	66.2%	χ^2=1.331, df=1	p≤0.25
Hours of sleep last night	6:25±1:22	6:45±1:27	F(1,212)=2.721	p≤0.10

[a] Mean ± standard deviation or percent (%) of total.
[b] Number of subjects varies slightly in both groups across variables due to missing values for some factors.
*Significant difference.

To evaluate whether possible relationships between family history status and the cortisol excretion measurements could be accounted for by the differences in socio-demographic and medical/health variables, three separate repeated measures ANOVAS were computed with the three cortisol collection times (work/home/sleep) as the within-subjects factor and: 1) race as the between-subjects factor, 2) smoking on assessment day as the between-subjects factor, and 3) alcohol consumption on assessment day as the between-subjects factor. None of the three repeated measures ANOVAS revealed a significant test of between-subjects effects or an interaction between the between-subjects factor and the within-subjects factor. As cigarette smoking is thought to stimulate the HPA axis (i.e., Gilbert et al., 2000), we further investigated the possible confounding effects of the higher number of participants smoking cigarettes on the assessment day in the FH+ group. T-tests with the dichotomous variable of smoking on assessment day as a factor and each of the four cortisol measures as dependent variables were computed among FH+ women only. None of the cortisol measures differed between smokers and non-smokers in the FH+ group (all $F[1,64]<.502$ and all $p>.48$). The non-significant associations with any of the cortisol measures for race, alcohol, and smoking suggest that these variables were unlikely to confound the group comparison, and were therefore dropped from further analyses.

The results from the two (group) X three (time) repeated measures ANOVA revealed a significant main effect of time using the Huynh-Feldt correction because of modest departures from 1 for epsilon ($F[1.83,388.95]=34.240$, $p\leq0.01$, partial $eta^2=0.138$) with higher values at work compared to the home and sleep microenvironments (see

Figure 1). There was no overall group difference on cortisol across the three microenvironments ($F[1,213]=1.368$, $p\leq0.24$). However, the interaction effect of Group by Time was significant ($F[1.83,388.95]=3.110$, $p\leq0.05$, partial $eta^2=0.014$), calling for further exploration of the data. One-way follow-up analyses indicated that the groups differed on cortisol collected during the work period. Women in the FH+ group had significantly higher values during work than women in the FH- group ($F[1,213]=3.845$, $p\leq0.05$, partial $eta^2=0.018$, see Figure 1); cortisol levels during the home period and the sleep period did not differ between the groups ($F[1,213]=0.271$, $p\leq0.60$, and $F[1,213]=0.195$, $p\leq0.66$, respectively), nor did the cortisol nadir levels (lowest on home or sleep) ($F[1,213]=0.180$, $p\leq0.67$), thus no support was found for an increase in trough levels of cortisol in women with family histories of breast cancer. However, consistent with increased responsivity to work stress, the FH+ group had a greater percentage increase from the cortisol nadir to work compared to the FH- group ($F[1,213]=3.945$, $p\leq0.05$, partial $eta^2=0.018$) (see Figure 2).

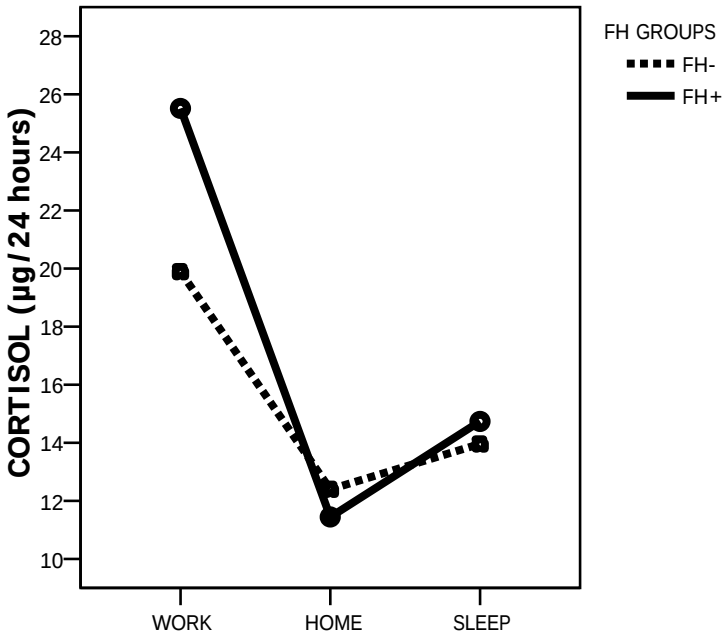

Figure 1. Comparison of urinary cortisol (µg/24 hours) excretion rates across the three contrasting daily microenvironments in women with at least one first-degree relative with breast cancer (**FH+,** N=74) and women without a first-degree relative with breast cancer (**FH-,** N=141).

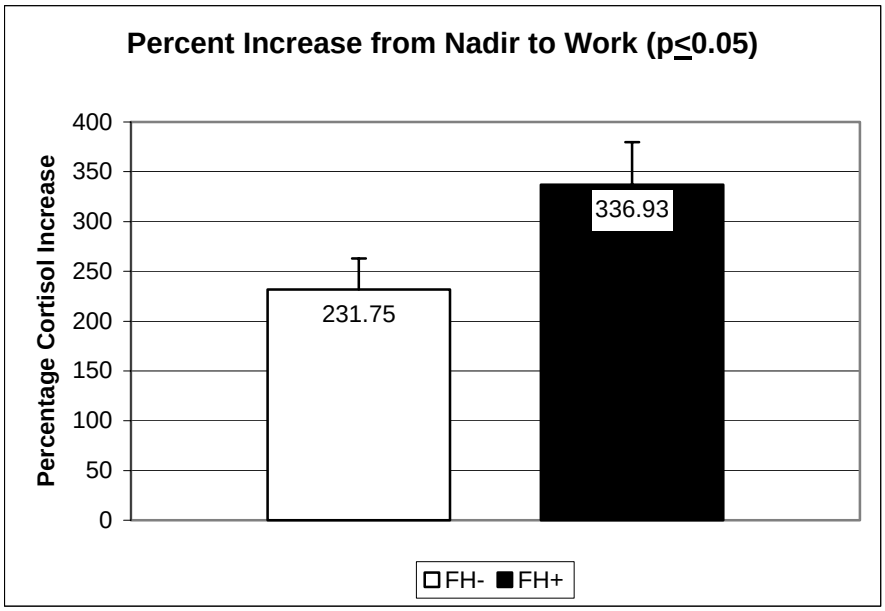

Figure 2. Comparison of the percent increase in urinary cortisol excretion rates from the cortisol nadir to work between women with at least one first-degree relative with breast cancer (FH+) and women without a first-degree relative with breast cancer (FH-).

Discussion

The present study investigated cortisol reactivity to day-to-day life in working women with different family histories of breast cancer. Consistent with study hypotheses, we found higher levels of urinary cortisol levels during a stressful period of the day (work) in women with at least one first-degree relative with breast cancer, compared to women without a first-degree relative with breast cancer. In addition, the percentage increase from the cortisol nadir (lowest value on either home or sleep) to work was higher in the FH+ group compared to the FH- group. These results are consistent with our previous experimental data indicating elevated cortisol responses to a standardized laboratory stressor in healthy women with family histories of breast cancer (Gold et al.,

2003). These results are also consistent with our recent finding of increased epinephrine excretion during work, supporting the view of a joint effort of the HPA axis and the SAM to mediate the stress response in women with positive family histories of breast cancer with catecholamines acting in concert with cortisol (Sapolsky, Romero, & Munck, 2000; Nicolaidis, 2002).

The possibility that there might be an elevated trough level of cortisol in the family history positive group compared to the family history negative group was not supported by our data. One possible explanation for this finding is that more frequent sampling (i.e., repeated saliva sampling) may be needed to fully characterize trough levels.

The literature on cortisol responsiveness has generally viewed acute stress responses and the output across the day as two separate phenomena that may be influenced by different factors. These factors include physiologic, genetic, and psychological variables. For example, while the circadian rhythms of HPA activity seem to be regulated mainly by high affinity mineralocorticoid receptors in the central nervous system, responses to acute stress appear to be regulated by low affinity glucocorticoid receptors (Dallman et al., 2000). Wust et al. (Wust et al., 2000) reported results from a twin study indicating that the cortisol waking response but not levels over the remainder of the day are under some genetic control. Chronic stress as a psychological factor that may influence the two components of the cortisol profile has been studied in animals and humans. In rats, different chronic mild stressors have shown to maintain peak corticosterone levels at normal ranges, while trough levels were increased (Spencer, Young, Choo, & McEwen, 1990; Dallman et al., 2000), even after stressor exposure had

stopped (Brennan et al., 2000). In humans, chronic stress, such as long-term unemployment, burnout, and work overload has been repeatedly shown to increase acute cortisol reactivity (i.e. Wust et al., 2000; Ockenfels et al., 1995; De Vente et al., 2003; Schlotz et al., 2004), but effects of chronic stress on cortisol levels over the day have yielded inconsistent results with some studies supporting an influence on trough cortisol levels (Cella et al., 1995; Linkowski et al., 1987; Powell et al., 2002; Van Cauter et al., 1996) and others not (Wust et al., 2000; Ockenfels et al., 1995).

Inconsistencies could stem from the wide variety of chronic stressors used across studies with different aspects of, for example, currency (ongoing vs. resolved) or duration including divorce/separation, unemployment, depression, mania, jet lag, and aging (Gump et al., 1999). It may be that the salience of the stressor influences the timing of cortisol responses, which would explain opposite results on cortisol secretion between unemployment and divorce as a chronic stressor, with unemployment influencing the stress response during the day and divorce influencing the home period (Powell et al., 2002).

It is not clear through which mechanisms family history of breast cancer alters reactivity to stressors. One intriguing possibility is that genetic factors account for or contribute to this enhanced responsivity. Supporting this possibility, Wust et al. (Wust et al., 2004) recently found that common polymorphisms in the glucocorticoid receptor gene may have modulating effects on the relation between psychosocial stress and HPA axis response. In addition to genetic factors, it would also be of interest in future research to examine shared environmental factors, which might differ between families with and without breast cancer.

The present study has limitations that must be recognized. First, the collection of urinary cortisol during three distinct time blocks is not a sensitive assessment of possible alterations in the diurnal pattern of cortisol and more frequent sampling is needed to fully characterize HPA axis alterations in women with positive family histories of breast cancer over the course of the day. It is possible that assessment of cortisol with multiple salivary samples collected across the day would reveal alterations in the circadian decline, and/or in the awakening response in these women. However, the naturalistic approach taken in the present study, measuring neurohumoral excretion rates in differing daily microenvironments outside the laboratory, did reveal significant effects of family history, consistent with previous research indicating that this approach captures variance as a consequence of the stressfulness of the environment (James et al., 1993; James et al., 1997).

Second, the present study did not include self-report assessments of perceived stress at work and at home. However, considerable evidence suggests that it is the work place that consistently elicits the strongest stress responses of the day (i.e., Brown et al., 2000; James et al., 1997; Kario et al., 2002). Further supporting that view, we found in a related study drawing from the same population of working women sampled here (unpublished observations) that self-reported anxiety levels at work were higher than in the home environment. In addition, it should be noted that one potential confounding source of home stress – children - (James et al., 1989) did not differ for women with and without family histories of breast cancer.

Considered together with our previous experimental (Gold et al., 2003; Valdimarsdottir et al., 2002) and naturalistic studies (James et al., 2004) the results of the

present study suggest that women with family histories of breast cancer have a pattern of increased psychobiological reactivity to acute stress. Additional research is now warranted to explore potential psychological mediators and moderators, as well as possible underlying biological mechanisms. Further research to explore the potential negative health consequences of this increased reactivity is also warranted given the literature linking increased reactivity, and particularly cortisol to health risk. As cortisol mediates effects ranging from induction of liver enzymes involved in energy metabolism to regulating the trafficking of immune cells and cytokine production (McEwen et al., 1999; Vanitallie, 2002; Chrousos, 2000), it is tempting to speculate that the higher levels of cortisol found during work in women with family histories of breast cancer may have negative health consequences.

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Cortisol responses to daily life stressors are increased among healthy population-risk women with higher levels of breast cancer specific distress[1]

Abstract

Objective: Survey studies have shown that women fear breast cancer more than any other disease. Psychological studies have found that breast cancer-specific distress levels are related to women's perception of their personal risk. The purpose of the present study was to explore possible biological consequences of higher risk perceptions and intrusive thoughts about breast cancer in women at population risk. Specifically, we hypothesized that women with higher perceived risk of breast cancer would also have more intrusive thoughts about breast cancer (Intrusion subscale of the Impact of Events Scale) which would constitute a background stressor sufficient to increase hypothalamus-pituitary-adrenal axis (HPA) responsivity to daily stress. *Methods:* HPA responses to an ordinary life stressor (work) were assessed in 141 employed women (age=37.2±9.2) who reported no first-degree relatives with breast cancer. Urinary cortisol excretion rates were assessed with timed sample collections at work (i.e., 11AM to 3PM), home (i.e., 6PM to 10PM), and during sleep (i.e., 10PM to 6AM). *Results:* Repeated Measures ANOVA revealed a significant Group by Time interaction with follow-up analysis indicating higher work cortisol levels in women with intrusions compared to women without

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intrusions. Regression analyses revealed a significant association between risk perceptions and intrusions ($p<0.001$), and regression analysis with intrusions and risk perceptions predicting work cortisol indicated a significant contribution of intrusions ($p<0.03$), but not risk perceptions ($p=0.932$). Identical results were apparent for delta cortisol levels. We conclude that overestimation of breast cancer risk is associated with higher levels of intrusive thoughts that can result in increased cortisol responsivity to daily stressors. This heightened responsivity could have long-term negative health implications.

Introduction

Breast cancer is the most frequently diagnosed malignancy among women in the Western world. It is the second leading cause of cancer mortality next to lung cancer in American women. Even though detection at earliest stages promises a 95% 5-year survival rate, 39,000 American women will die of the disease this year (ACS, 2004).

"Perhaps no other disease summons the kind of dread in women than that evoked by even the mention of breast cancer" (Wear, 1993, p.82). In J. S. Olson's a book entitled "Women, cancer, and history" (Olson, 2002) breast cancer is labeled as "a horror known to every culture in every age… fear of the disease [has not] eased much over the years or over centuries and millennia…" (front flap and prologue). Consistent with these citations a national survey of 1045 women (Spittle & Morgan, 1999) revealed that breast cancer is one of the most feared of all diseases.

Fear of breast cancer may be evoked by the media. While breast cancer was surrounded by secrecy until the 1980s, an increase in consumer advocacy and media coverage has brought the condition to the attention of the public (Baum, 2000), and an emphasis on atypical cases of early-onset breast cancer in the media (Burke, Olsen, Pinsky, Reynolds, & Press, 2001) has made the adverse side effects of treatments, including disfiguring surgery, radiation and chemotherapy a salient feature of the disease in people's minds.

Although media coverage and campaigns have increased awareness of breast cancer among women, the information provided does not necessarily translate into an accurate understanding of personal risk. Several studies have demonstrated that women

greatly overestimate their risk of getting breast cancer. For example, McGregor and colleagues (McGregor et al., 2004) recently reported mean perceived risk levels of 37.2% in a large, community-based sample despite the fact that the actual risk computed with the Gail model for risk appraisal (Gail et al., 1989) was consistent with published risk estimates of 10.5% for a community sample. This result is consistent with an earlier study by Erblich and colleagues (Erblich, Montgomery, Valdimarsdottir, Cloitre, & Bovbjerg, 2003) who reported a mean perceived lifetime risk of 25.8 in women without family histories of breast cancer corresponding to an estimated actual risk of 11% according to the Claus breast cancer risk assessment model (Claus, Risch, & Thompson, 1994).

The finding that women overestimate their risk of breast cancer furthermore suggests that women not only fear the disease but that they may also experience high levels of cancer-specific distress, such as frequent cancer worries, intrusive thoughts about breast cancer, and avoidance regarding the disease. In a number of studies, elevated levels of breast cancer risk perceptions have been associated with elevated levels of cancer-specific distress among women with family histories of the disease (Erblich, Bovbjerg, & Valdimarsdottir, 2000; Lerman et al., 1993; Zakowski et al., 1998; Kash, Holland, Halper, & Miller, 1992) and among women in random community samples (Lipkus et al., 2000; McGregor et al., 2004).

The present study aims to investigate whether frequent cancer-specific distress symptoms in women at population risk for the disease have biological consequences. Specifically, we propose that intrusive thoughts about breast cancer may be a background stressor sufficient to elicit increased cortisol levels in response to acute stress, an

indication of an alteration of hypothalamic-pituitary-adrenal (HPA) axis function. Considerable research in animals and humans provides compelling evidence that acute stress responses are increased in the presence of a background stressor (Gump & Matthews, 1999). In humans, enhanced acute cardiovascular and neuroendocrine reactivity to unrelated stress seems to be particularly true for background stressors that are ongoing, long-term, frequent, and important (Gump et al., 1999), albeit with fewer and more variable results for HPA axis measures (Matthews, Gump, & Owens, 2001).

In support of a link between intrusive thoughts and elevated cortisol secretion, research in individuals living in proximity to the Three Mile Island nuclear accident revealed a relationship between persistent thought intrusion about the accident and elevations in urinary free cortisol (Baum, Cohen, & Hall, 1993), and more recently, Lutgendorf et al. (Lutgendorf, Reimer, Schlechte, & Rubenstein, 2001) reported an association between intrusive thoughts and higher resting cortisol levels concurrently and prospectively among older adults experiencing house relocation.

While there are several studies on perceived breast cancer risk and emotional and cognitive correlates (Erblich et al., 2000; Lerman et al., 1993; Zakowski et al., 1998; Kash et al., 1992), as well as studies evaluating interventions to lower breast cancer risk perceptions (Bowen, Burke, McTiernan, Yasui, & Andersen, 2004), there are no studies, to our knowledge, that have investigated possible biological consequences of increased risk expectancies for breast cancer and intrusions about the disease. Furthermore, we are not aware of any studies that have investigated the relationship between intrusions and cortisol across three microenvironments (work, home, and sleep) where work was operationally defined as the stressor condition, based on earlier studies indicating that it is

the work place that consistently elicits the strongest stress responses of the day (Brown & James, 2000; James & Brown, 1997; Kario, James, Marion, Ahmed, & Pickering, 2002).

The goal of the present study is to investigate perceived breast cancer risk, intrusive thoughts about breast cancer and cortisol reactivity in women at population risk for breast cancer. Specifically, we hypothesize that a) women with higher perceived risk of breast cancer will have more intrusions about breast cancer, b) intrusions about breast cancer will be related to increased HPA responsivity to work stress, and c) intrusions will be related to higher delta cortisol levels (work-nadir (sleep or home)).

Method

Design and Subjects

Cortisol responses were investigated under daily life conditions dividing the workday of employed women into three different microenvironments: work, home and sleep. Urinary cortisol excretion rates were compared across these contrasting daily conditions, as previously described (Dettenborn et al., 2004; James, Berge-Landry, Valdimarsdottir, Montgomery, & Bovbjerg, 2004).

The participants of the study were a sample of 141 healthy women without a personal or first-degree family history of breast cancer who were employed at three major medical centers in New York City. This sample is part of a larger sample which we reported on previously, where we could demonstrate heightened cortisol responses to daily stress in women at familial risk for breast cancer compared to women at population risk for the disease (Dettenborn et al., 2004). Women were recruited through

advertisement and had agreed to participate in a study of women with different family histories of breast cancer. The response rate of those women who responded to the ads was above 90%. The following exclusion criteria for participation in this study were applied: 1) no first-degree family history of breast cancer, 2) not English speaking, 3) a history of HIV, cancer or abnormal breast exams (including abnormal breast biopsy or abnormal mammogram), 4) medication use other than birth control pills, and 5) participation in any other research study that could potentially affect our study variables. The study was conducted under institutional review board approval and all participants signed informed consent. Based on their self-reported family histories of cancer women in this study were specifically selected as fulfilling the criteria of not having had a first-degree relative with breast cancer (n=141). For this study, only women with complete data on all three cortisol measurements were included in the analyses.

Procedures

On the morning of the study day, participants met with trained research personnel, completed informed consent, demographic data, medical history, and the remaining procedures were explained to them.

Perceived Breast Cancer Risk. Perceived breast cancer risk was assessed with one item ("How likely do you think it is that you will develop breast cancer in your lifetime?"). The response options to this question ranged from 0 (0%) to 100 (100%). This is one of the most widely used formats to assess perceived disease risk.

Intrusive thoughts about breast cancer. Intrusive thoughts about breast cancer were assessed using the Impact of Event Scale (IES) (Horowitz, Wilner, & Alvarez,

1979), which is a 15-item self-report instrument measuring intrusive thoughts (7 items), avoidant behaviors (7 items), and interference with daily activities (1 item) over the last 7 days in relation to a specified stressful event, here breast cancer. Responses are recorded on a Likert scale ranging from 0 ("not at all") to 5. Test-retest reliability and external validity for the IES are high (Horowitz et al., 1979). In this sample, the Cronbach's alpha of the intrusion subscale was 0.87.

Anthropometrics. Anthropometric measurements including weight and height to calculate body mass index (BMI) were taken.

Urinary cortisol sampling. Participants were provided with a urine collection bottle for the work period at one of the three medical centers where all hospital workers were recruited from and all procedures were explained to them. They were contacted again the same day and two more urine collection bottles were provided to them for the home period and the sleep period. These specimens were returned to the research laboratory the next morning.

The specific urine collection procedures were based on those described by James et al. (James, Schlussel, & Pickering, 1993) and have been reported previously by us (Dettenborn et al., 2004). Briefly, the first urine specimen at work was not collected but indicated the beginning of the work urine collection period for the study. The participants then collected their urine in the provided 3-liter polyethylene bottle with preservative (0.5 g of sodium metabisulphite) across a block of time (i.e., mean of four hours for the work block). The time of the first and last sample was noted. The same procedures were followed for the home and sleep sampling periods resulting in samples for the three distinct daily environments defined as work (approximately 11AM to 3PM), home

(approximately 6PM to 10PM), and sleep (approximately 10PM to 6AM). The total volume of each sample and the length of time of the collection were recorded for each collection period, so that cortisol values could be corrected by volume and expressed as rate of excretion (µg/24 hours) (see Appendix D). In preparation for the cortisol assay, a 5 ml aliquot was taken from each sample and stored at –60 ^{0}C for future batch assay. Concentrations of cortisol were determined using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA) with a sensitivity of 0.2 µg/dL and inter- and intra-assay coefficients of variance below 7% for all analyses.

Statistical analysis

All variables were checked for outliers and normality. Transformations were conducted where appropriate: The total listwise deletions due to extreme values on any of the three cortisol measures were 2 following an algorithm of four standard deviations above the mean. To further improve distributions and reduce kurtosis (3.5, 7.3, and 4.5 for work cortisol, home cortisol, and sleep cortisol, respectively), a square root transformation was applied to the cortisol values. We further determined the cortisol nadir for each participant, the lowest level of the day (home or sleep period) and computed the cortisol delta subtracting the nadir from the work level.

Total values on the intrusion subscale of the IES were highly kurtotic (Kurtosis=6.23; Mean=3.55$\pm$5.96; Median=1), resulting in dichotomized values using the median split (0 and $\geq$1) (see Appendix B). For consistency, for a model with risk perceptions and intrusions included, risk perceptions were also transformed into high and low estimates using the median split (Median=30).

Group differences between women with and without intrusions were evaluated using ANOVA for continuous variables and Chi-Square for categorical variables. Variables with significant differences between the intrusion groups were entered as covariates. Repeated measures ANOVA with the three cortisol time points and follow-up one way ANOVAs were computed. Regression analyses predicting the cortisol time points with significant differences between the groups from intrusions and perceived risk were conducted, as well as identical analysis with the cortisol delta as the outcome.

Results

Table 1 shows selected socio-demographic characteristics of the sample. Overall, the sample was quite diverse: 53.3% of the women were white, 65.2% of the women were single, 66.2% held a college degree, and 90.8% were full-time employed at one of the three medical centers at the time of the study. The remaining 9.2% of women were hospital workers in less than a full-time employment situation. No significant demographic differences were observed between the intrusion groups.

Table 1: Socio-demographic characteristics of the study sample.[a]

Socio-demographic characteristics	**Intru=0 n=59** [b]	**Intru≥1 n=82**	**Statistical test value**	**p-value**
Mean age	36.8±8.2	37.4±9.9	F(1,135)=0.158	p<0.69
Ethnicity			χ^2=0.584, df=1	p<0.45
-Hispanic	10.5%	15.0%		
Race			χ^2=0.584, df=2	p<0.75
-White	56.1%	51.3%		
-Black	35.1%	36.3%		
-Other	8.8%	12.5%		
Marital Status			χ^2=0.032, df=1	p<0.86
-currently married	35.6%	34.1%		
Education			χ^2=4.707, df=2	p<0.10
	25.0%	38.8%		
-some college or less	42.9%	26.3%		
-College degree	32.1%	35.0%		
-Graduate degree				
Employment			χ^2=0.067, df=1	p<0.80
-full time	91.5%	90.2%		

[a] Mean + standard deviation or percent (%) of total. FH- Women without a first-degree relative with breast cancer; FH+ Women with at least one first-degree relative with breast cancer.

[b] Number of subjects varies slightly in both groups across variables due to missing values for some factors.

* Significant difference.

As shown in Table 2, group differences in medical history/health-related variables were found only for perceived breast cancer risk (F[1,139]=16.547, p<0.001), which was expected (Hypothesis 1).

Table 2: Medical history variables of the study sample [a]

Medical History	**Intru=0 n=59** [b]	**Intru≥1 n=82**	**Statistical test value**	**p-value**
BMI	25.9±6.1	27.2±7.5	F(1,129)=1.241	p<0.27
Pre-menopausal	84.7%	75.6%	χ^2=1.753, df=1	p<0.19
Children (>1)	40.7%	45.1%	χ^2=0.276, df=1	p<0.60
Birth control pills ever	63.8%	70.9%	χ^2=0.772, df=1	p<0.38
HRT ever	1.8%	3.8%	χ^2=0.501, df=1	p<0.48
Cortisone regularly	7.0%	10.3%	χ^2=0.427, df=1	p<0.51
Smoked on assessment day	4.3%	4.2%	χ^2=0.002, df=1	p<0.96
Drank alcohol on assessment day	9.1%	2.6%	χ^2=2.694, df=1	p<0.10
Drank caffeine on assessment day	59.6%	56.8%	χ^2=0.112, df=1	p<0.74
Hours of sleep last night	6:18±1:19	6:29±1:23	F(1,138)=0.533	p<0.47
Perceived BC risk	24.2±19.2	39.0±24.0	F(1,139)=16.547	p<0.001*

[a] Mean + standard deviation or percent (%) of total.

[b] Number of subjects varies slightly in both groups across variables due to missing values for some factors.

*Significant difference.

There was no overall group difference on cortisol across the three microenvironments (p=0.11). However, as expected, there was a significant effect of time (F[2,278]=13.601, p<0.001), and the interaction effect of Group by Time was significant (F[2,278]=5.723, p<0.05) calling for further exploration of the data (Figure 1).

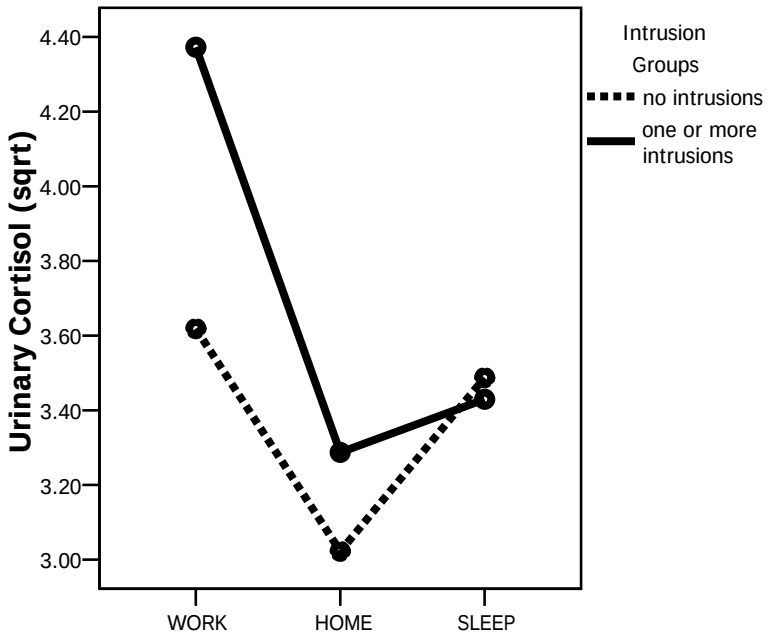

Figure 1. Urinary cortisol at work, home, and sleep among women at population risk for breast cancer with and without intrusions for breast cancer.

One-way follow-up analyses indicated that the intrusion groups differed on cortisol collected during the work period ($F[1,139]=5.831$, $p<0.02$) with higher work cortisol values for women who experienced at least one intrusive thought during the past week. The home and sleep period did not differ ($F[1,139]=2.410$, $p=0.31$, and $F[1,139]=0.055$. $p=0.81$, respectively). Additionally, the cortisol delta (work cortisol minus nadir cortisol) was significantly higher in the intrusion group ($F[1,139]=4.204$, $p<.05$) (Figure 2).

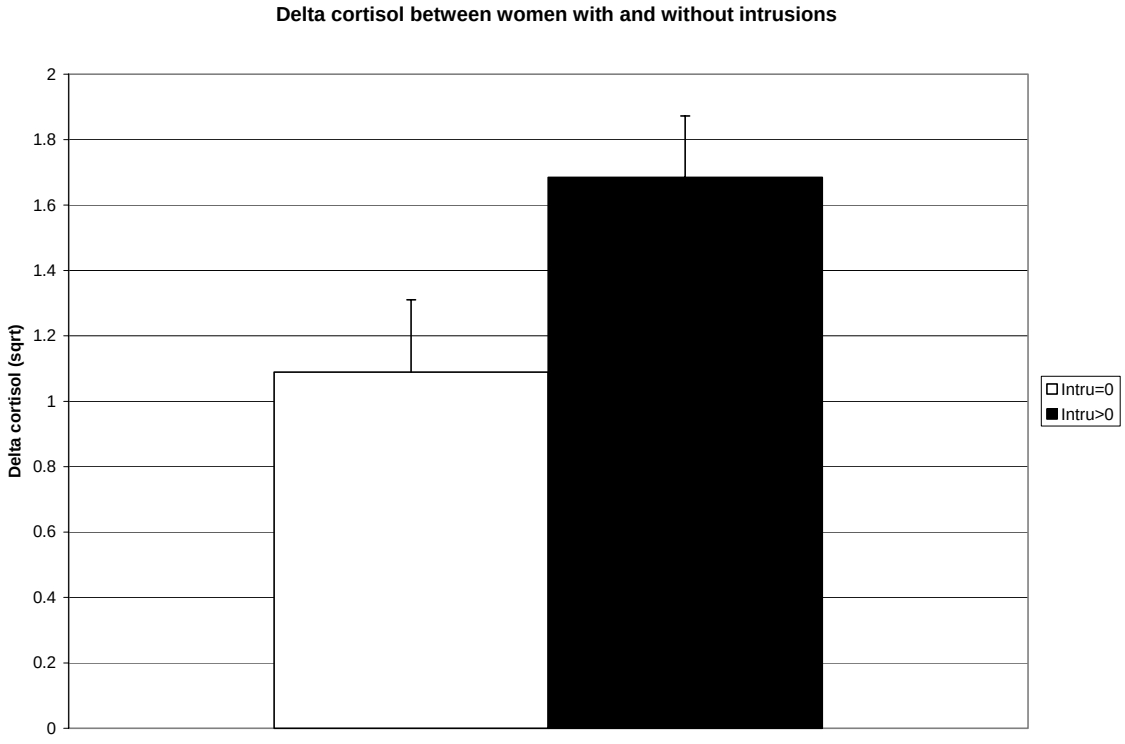

Figure 2. Delta cortisol levels (with standard error bars) among women at population risk for breast cancer with and without intrusions for breast cancer.

Regression analyses with intrusions (high vs. low) and risk perceptions (high vs. low) predicting work cortisol levels indicated a significant contribution of intrusions ($p<0.03$), but not risk perceptions ($p=0.932$). The same contribution of intrusions ($p=0.05$), but not risk perceptions ($p=0.95$) was apparent when predicting the delta value (work minus nadir). Including education and alcohol on assessment day as covariates (as they showed trends for group differences) did not change the findings.

Discussion

The goal of the present study was to investigate whether perceived breast cancer risk and intrusive thoughts about breast cancer have biological consequences, such as increased cortisol reactivity, in women at population risk for breast cancer. Consistent with study hypotheses, we found that women with intrusive thoughts about breast cancer estimate their risk of developing breast cancer during their lifetime as significantly higher compared to women without intrusions about the disease. Intrusions, but not risk perceptions were related to cortisol levels during work, supporting previous reports on increased acute stress responses in the presence of a background stressor (Gump et al., 1999).

While different categorizing of the IES has been applied previously (Horowitz, 1982), the use of a dichotomized intrusion scale with no intrusive symptoms vs. some intrusive symptoms in this study has only been done once before, to our knowledge (Zakowski, Valdimarsdottir, & Bovbjerg, 2001). The intrusion subscale of the IES assesses the frequency of each of seven intrusive symptoms during the past week with a score of 1 being rarely, 3 being sometimes, and 5 being often for each symptom. While the IES is one of the most widely used self-report instruments of posttraumatic stress and has been used across many different trauma samples (Joseph, 2000), here, it is used in a non-traumatized sample with the stressor of a highly prevalent but curable disease, if detected early. Therefore, distributions and cut-off values need to be reconsidered in this sample. The specific idea of the current analysis was to assess intrusive thoughts about breast cancer as a background stressor sufficient to increase cortisol reactivity to an

unrelated stressor. A score of one or above on the intrusion subscale of the IES, that is at least one intrusive symptom experienced rarely during the past week, is, in our opinion, an appropriate criterion. In our sample, 47.8% and 34.2% have scores higher than 3 and higher than 5, respectively, indicating a fair amount of background stress. As Huizinga and colleagues point out (Huizinga et al., 2005), the IES is considered to be an index of stress response symptoms and not an index of PTSD symptoms, for which it has been used widely, because it provides no information about hyperarousal, which is a criterion for the DSM diagnosis of PTSD. Therefore, we consider it an appropriate use for our purpose of assessing background stress levels.

The present finding indicates that in women at population risk for breast cancer intrusive thoughts about breast cancer are a background stressor strong enough to elicit increased levels of cortisol during work. Gump and colleagues state that, background stressors that are ongoing, long-term, frequent, and important are most likely to enhance acute cardiovascular and neuroendocrine reactivity to unrelated stress (Gump et al., 1999), albeit with fewer and more variable results for HPA axis measures (Matthews et al., 2001). The threat of breast cancer falls into these categories as a) it is the most frequently diagnosed cancer in women making this an ongoing and long-term stressor, b) it is the second leading cause of cancer mortality in American women (ACS, 2004) characterizing this stressor as one of high importance, and c) an increase in consumer advocacy and media coverage has brought the condition to the attention of the public (Baum, 2000) providing frequent reminders of the disease threat. In concordance with the objective threatening and stressful nature of breast cancer, women's appraisal of the event is the most fear out of all diseases as identified by a national survey of 1045 women

(Spittle et al., 1999). Last, while breast cancer can be detected at earliest stages with a significant reduction of mortality rates, modifiable risk factors for breast cancer are not well established, thus making successful prevention of the disease difficult and uncontrollability, a main component for stress experiences (Dickerson & Kemeny, 2004), high.

The present study has limitations that must be recognized. Women in this study were recruited through advertisement as taking part in a larger study of women with and without family histories of breast cancer and came to the department of oncological sciences for their study visit. We can not rule out that women who decided to participate in this study were more distressed about breast cancer than the general population. Furthermore, taking part in a breast cancer study may have heightened their awareness and distress about breast cancer on a temporary basis. Interestingly, however, only 26.8% of women in our sample indicated that they had thoughts about breast cancer on any of the three days preceding the visit and on the day of the visit.

Future research should address precursors and correlates of intrusive thoughts about breast cancer in population risk women. It is also warranted to study the role of media coverage through assessment of this phenomenon in different countries. We have to be open to the possibility that an increase of awareness of the disease with the goal of reducing mortality through cancer screening behavior may have unwarranted negative health implications through an increase in stress reactivity.

In sum, the present study suggests that intrusive thoughts about breast cancer are pronounced among women with no affected first-degree family members, to the point

that they correlate with increased HPA reactivity, a condition that has been shown to lead to detrimental health effects (McEwen & Seeman, 1999; McEwen, 2000).

Elevated work-stress cortisol responses in women at familial risk for breast cancer: Predicted by intrusions about breast cancer?[1]

Abstract

Objective: Healthy women with family histories of breast cancer have stronger cortisol responses to daily stressors. As these women also report higher levels of perceived risk and intrusions about breast cancer, these factors were examined as predictors of the stronger cortisol responses to daily stressors. *Method:* Participants were 185 healthy premenopausal working women with first-degree relatives diagnosed before (HiFR=37, high familial risk) or after age 50 (LoFR=27, low familial risk), or without cancer in first-degree relatives (NoFR=121, no familial risk). Participants completed self-report measures of perceived lifetime breast cancer risk (0-100%), intrusive thoughts and avoidance about breast cancer (Impact of Event Scale), negative affect, and general distress. Urine samples were collected for assessment of cortisol responses. *Results:* Repeated measures ANOVA revealed a Group by Time interaction ($p<0.05$) with HiFR having higher ($p<0.03$) urinary cortisol levels than NoFR during work, but not at home or during sleep, while cortisol levels in LoFR women were not different at any time. HiFR also showed higher levels of perceived breast cancer risk, intrusions, and avoidance compared to both NoFR and LoFR ($p<0.001$), but not negative affect or general distress ($p>0.6$). Perceived risk and avoidance about breast cancer were not related to cortisol

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responses ($p>0.17$), but intrusions were ($p<0.01$). Including breast cancer risk perceptions, avoidance, and intrusions along with group in the analysis indicated a significant contribution of intrusions ($p<0.02$), but not risk perceptions ($p=0.52$) or avoidance ($p=0.98$), and a no longer significant effect of group ($p=0.12$). However, the Sobel test for mediation was not significant. Separate analyses within each of the groups indicated significant relations between intrusions and work cortisol among NoFR ($p<0.02$), but not among LoFR ($p=0.49$), or HiFR ($p=0.43$). Results provide conclusive evidence of intrusion effects, but not of mediation of group effects.

Introduction

Women with at least one first-degree relative with breast cancer, which increases a woman's lifetime risk of developing the disease by up to four times (Evans & Lalloo, 2002), have been found to have stronger psychobiological reactions to acute stressors in both experimental and naturalistic studies (Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003; James, Berge-Landry, Valdimarsdottir, Montgomery, & Bovbjerg, 2004; Valdimarsdottir et al., 2002). Most recently, we have reported that healthy working women at familial risk for breast cancer have higher levels of urinary cortisol during a stressful period of the day (work) compared to women without a first-degree relative with breast cancer (Dettenborn et al., 2004). These results demonstrate increased cortisol reactivity to acute stress under daily life conditions in women at familial risk for breast cancer compared to women at population risk for the disease. These findings are entirely consistent with our previous experimental data, which revealed elevated cortisol responses to a standardized laboratory stressor in healthy women with family histories of breast cancer compared to women without family histories of the disease (Gold et al., 2003). Further consistency exists with a recent report by Cohen and colleagues of higher plasma cortisol levels (a surrogate measure of acute cortisol reactivity) in daughters of breast cancer patients (Cohen, 2002). In sum, experimental and naturalistic studies support heightened acute cortisol responses in healthy women at familial risk for breast cancer compared to women at population risk for the disease. These findings are congruent with recent theorizing by Gump and colleagues (Gump & Matthews, 1998;

Gump & Matthews, 1999) that acute stress responses are enhanced in individuals contending with ongoing background stressors.

The most robust findings in the psychological literature on healthy women with family histories of breast cancer have been higher perceived risk of the disease and higher levels of intrusions related to breast cancer. A recent meta-analytic review of twelve studies examined the relationship between having a positive family history of breast cancer and perceived breast cancer risk and found that women with family histories of breast cancer were significantly more likely to perceive their risk of developing the disease as higher than that of other women (total N=70,660, g=0.88, 95% CI 0.87-0.89) (Katapodi, Lee, Facione, & Dodd, 2004). The result of higher perceived breast cancer risk in women with family histories of the disease is consistent across the twelve studies, despite the fact that nine studies used a verbal and/or comparative, Likert-type scale to measure perceived risk, a type of measurement that is more likely to produce an optimistic bias (Lipkus et al., 2000). The second robust finding differentiating women at familial risk for breast cancer from women at population risk for the disease is a higher occurrence of intrusions about breast cancer among the former (Baider, Ever-Hadani, & Kaplan De-Nour, 1999; Valdimarsdottir et al., 1995; Zakowski et al., 1997) even long after the diagnosis of breast cancer in their first degree relatives (mean 14.4 years) (Erblich, Montgomery, Valdimarsdottir, Cloitre, & Bovbjerg, 2003).

In a separate literature, intrusions have been shown to be associated with hypothalamus-pituitary-adrenal (HPA) axis measures more generally (Antoni et al., 1990; Baum, Cohen, & Hall, 1993; Lutgendorf, Reimer, Schlechte, & Rubenstein, 2001). For example, Baum et al. found in individuals living in proximity to the Three Mile Island

nuclear accident a relationship between persistent thought intrusion about the accident and elevations in 15h overnight urinary free cortisol (Baum et al., 1993). More recently, Lutgendorf et al. reported an association between intrusive thoughts and higher plasma free cortisol levels concurrently and prospectively among older adults moving to congregate living facilities (Lutgendorf et al., 2001). It has been suggested that repeatedly contending with an ongoing stressor (which might be indicated by experiencing frequent intrusions) would lead to sustained vigilance for possible threat which could contribute to chronic or repeated arousal, as well as priming for stronger responses to unrelated acute stressors (Gump et al., 1998). To explore the specificity of intrusions in relationship to acute cortisol responses, we also explored other more general psychological variables previously shown to differ between familial risk groups in some studies: avoidance (Erblich et al., 2003; Zakowski et al., 1998), negative affect (Erblich, Bovbjerg, & Valdimarsdottir, 2000; Erblich et al., 2003), and general distress (Baider et al., 1999; Valdimarsdottir et al., 1995).

The present study aimed to investigate psychological factors that could explain previously demonstrated increases in acute cortisol response in women at familial risk for breast cancer. Because familial risk estimates are highest among premenopausal women with first-degree relatives diagnosed at premenopausal age, we limited the present analyses to women below 50 years of age and explored urinary cortisol levels at work, home, and sleep and possible psychological mediators among healthy premenopausal women at population risk for breast cancer compared to low and high familial risk women (based on the age of their relative at diagnosis) (Dite et al., 2003). Based on the literature reviewed above, we hypothesized: 1) that work cortisol levels would be most

pronounced for women with first-degree relatives diagnosed at premenopausal age (high familial risk) (Dite et al., 2003), less pronounced for women with first-degree relatives diagnosed at postmenopausal age (low familial risk), and the least pronounced for women without first-degree relatives with breast cancer (population risk), 2) that intrusions about breast cancer would mediate the relationship between familial risk status and work-stress cortisol responses. We hypothesized that an association between breast-cancer specific distress and cortisol would be specific to intrusions about the disease and would not be apparent for perceived breast cancer risk, avoidance about breast cancer, negative affect, and general distress, variables that we predicted to differ between familial risk groups.

Method

Design and Subjects

Cortisol responses were investigated under daily life conditions, with the use of the same protocol as previously described (Dettenborn et al., 2004), where work was operationally defined as the stressor condition, based on earlier studies indicating that it is the work place that consistently elicits the strongest stress responses of the day (i.e., (Brown & James, 2000; James & Brown, 1997; Kario, James, Marion, Ahmed, & Pickering, 2002).

The subjects of the study were a sample of 185 healthy premenopausal women without a personal history of breast cancer who were employed at three major medical centers in New York City. Women were recruited through advertisement and agreed to

participate in a study of women with different family histories of breast cancer. The response rate of those women who responded to the ads was above 90%. The following exclusion criteria for participation in this study were applied: 1) not English speaking, 2) a history of HIV, cancer or abnormal breast exams (including abnormal breast biopsy or abnormal mammogram), 3) medication use other than birth control pills, and 4) participation in any other research study that could potentially affect our study variables. The study was conducted under institutional review board approval and all participants signed informed consent. Based on their self-reported family histories of cancer women in this study were classified into (1) not having a first-degree relative with breast cancer (NoFR, n=121), (2) having a first-degree relative with breast cancer diagnosed at postmenopausal age ≥ 50 years (LoFR, n=27; low familial risk), and (3) having a first-degree relative with breast cancer diagnosed at premenopausal age (HiFR, n=37; high familial risk). For this study, only women with complete data on cortisol measurements were included in the analyses.

Procedures

On the morning of the study day, participants met with trained research personnel, completed informed consent, demographic data, medical history, and the remaining procedures were explained to them.

Breast Cancer Risk Perceptions. Perceived breast cancer risk was assessed with one item ("How likely do you think it is that you will develop breast cancer in your lifetime?"). The response options to this question ranged from 0 (0%) to 100 (100%). In this sample, the median split was at 40, and the range from 0 to 100. This numerical scale

is one of the most widely used formats to assess perceived disease risk next to a verbal scale (Katapodi et al., 2004).

Intrusive thoughts and avoidance about breast cancer. Intrusive thoughts about breast cancer and avoidance were assessed using the Impact of Event Scale (IES) (Horowitz, Wilner, & Alvarez, 1979), which is a 15-item self-report instrument measuring intrusive thoughts (7 items), avoidant behaviors (7 items), and interference with daily activities (1 item) over the last 7 days in relation to a specified stressful event, here breast cancer. Responses are recorded on a Likert scale ranging from 0 ("not at all") to 5 ("often"). Test-retest reliability and external validity for the IES are high (Horowitz et al., 1979). In previous research conducted reliability analyses of the IES among women at increased risk for hereditary breast cancer established satisfactory test-retest reliability ($r=0.75$, 0.78, and 0.80 for the intrusion and avoidance subscales and total scale, respectively) and internal consistency (Cronbach's $\alpha=0.88$, 0.84, and 0.91 for the intrusion and avoidance subscales and total scale, respectively), as well as preliminary support for the concurrent and discriminative validity of the IES among women at increased risk of developing breast cancer (Thewes, Meiser, & Hickie, 2001). In this sample, Cronbach's alpha for the intrusion and avoidance subscales was 0.86 and 0.85, respectively. Consistent with previous IES analyses (Zakowski, Valdimarsdottir, & Bovbjerg, 2001), participants were categorized as having had intrusions about breast cancer during the past week (n=116) and not having had intrusions about breast cancer during the past week (n=69). The total scores for intrusion and avoidance ranged from 0 to 29 and 0 to 34, respectively (see Appendix B).

General Distress. The Brief Symptom Inventory (BSI) (Derogatis & Spencer, 1982) assesses psychological symptoms associated with general distress on nine subscales and one summary score, the General Severity Index (GSI), which was used for the purpose of the present analyses. Discomfort from 53 symptoms over the last 7 days is assessed on a scale from 0 ("not at all") to 4 ("extremely"). Possible GSI scores range from 0 to 4 averaged across the 53 items. Internal consistency alpha coefficients were reported as 0.71 to 0.85, and test-retest reliability coefficient as 0.90. In this sample Cronbach's alpha was 0.95, the median split at 0.23, and the range of GSI scores from 0 to 2.13.

Negative Affect. The Profile of Mood States (POMS) (McNair, Lorr, & Droppelman, 1971) was used to assess negative affect over the last 7 days. The scale consists of 65 affect adjectives rated on a 5-point Likert scale from 0 ("not at all") to 4 ("extremely"). As in other studies (Stanton, Danoff-Burg, & Huggins, 2002), five scales assessing negative mood (anger, depression, tension, fatigue, confusion) were summed to create a POMS Distress index with a possible range from 0 to 136, which has previously been reported to have good internal consistency (Cronbach's α=0.90-0.94). In this sample Cronbach's alpha was 0.94, the median split at 10, and the range of the POMS Distress index from 0 to 119.

Anthropometrics. Anthropometric measurements including weight and height to calculate body mass index (BMI) were taken.

Urinary cortisol sampling. Participants were provided with urine collection bottles for the work, home, and sleep period at one of the three medical centers where all

hospital workers were recruited from and all procedures were explained to them. They were contacted again the same day for collection of the specimen.

The specific urine collection procedures were based on those used extensively by James and colleagues (James, Schlussel, & Pickering, 1993), as previously described (James et al., 2004; Dettenborn et al., 2004). In short, the first urine specimen at work was not collected but indicated the beginning of the work urine collection period for the study. The participants then collected their urine in the provided 3-liter polyethylene bottle with preservative (0.5 g of sodium metabisulphite) across a block of time (approximately 11AM to 3PM). This preservative, widely used for this purpose has no known affect on the cortisol assay employed here (Cohen, de Moor, Devine, Baum, & Amato, 2001; Doering et al., 2000; Glover & Poland, 2002). The time of the first and last sample was noted. Total volumes of each sample and the length of time of the collection were recorded for each collection period, and cortisol values were corrected by volume and expressed as rate of excretion (μg/24 hours). Potential collection confounding was addressed by preliminary analyses that compared urinary volumes between the three groups for the time blocks: no significant differences were revealed (see Appendix D). We chose not to assess creatinine because urinary creatinine levels are highly variable both within and between individuals and thus cannot provide a reliable indicant of the adequacy of urinary sampling, or serve as a valid referent for other urinary metabolites (i.e., James et al., 1988; Vestergaard & Leverett, 1958). In preparation for the cortisol assay, a 5 ml aliquot was taken from each sample and stored at –60 ^{0}C for future batch assay. Concentrations of cortisol were determined using radioimmunoassay (Diagnostic

Products Corporation, Los Angeles, USA) with a sensitivity of 0.2 μg/dL and inter- and intra-assay coefficients of variance below 7% for all analyses.

Statistical Analyses

For outliers on the dependent variable of cortisol an algorithm of four standard deviations above the mean for each study group was established, so that outliers were sought separately within each group (NoFR/LoFR/HiFR grouping). The total listwise deletions due to extreme values were six. Square root transformations were computed to normalize distributions. Group differences between NoFR, LoFR, and HiFR were evaluated using ANOVA for continuous variables and Chi-Square Test for categorical socio-demographic and medical history variables. To rule out the possibility that participation in a breast cancer study had an effect on any of the psychological scales, responses to the question "Have you had thoughts about breast cancer today/yesterday/2 days ago/3 days ago" were also explored. To evaluate whether possible relationships between familial risk status and the cortisol measurement could be accounted for by differences in socio-demographic and/or medical/health variables, those with differences were introduced as between-subjects factors in univariate ANOVAs with work cortisol as the dependent variable.

Due to extreme distributions on the IES Intrusion Scale, participants were categorized as having had intrusions about breast cancer during the past week (n=116) and not having had intrusions about breast cancer during the past week (n=69), which is consistent with previous IES analyses (Zakowski et al., 2001). The same approach was used for the IES Avoidance Scale resulting in 78 women without avoidant behaviors and

107 women with avoidant behaviors. All other scales were used as median splits (perceived breast cancer risk, negative affect, and general distress). Group differences on dichotomized psychological variables were evaluated using logistic regression with simple contrasts using NOFR as the reference group.

Repeated measures ANOVA with familial risk group as the between-subject factor and the three cortisol measurements as the within-subjects factor was conducted. Univariate follow-up ANOVAs were computed to reveal group differences at each time point. Separate repeated measures ANOVAs with any of the psychological variables with group differences as fixed factors and cortisol as the dependent variable were conducted. Univariate follow-up ANOVAs were computed to reveal relations between the psychological variable and cortisol at each time point. To investigate mediation, the cortisol time point with group differences and relations to a psychological variable was introduced as dependent variable in a univariate ANOVA with familial risk group and the psychological variables as factors (Baron & Kenny, 1986; MacKinnon et al., 2002).

Results

Familial Risk Group Differences: Socio-Demographic and Medical History Variables

Table 1 shows selected socio-demographic characteristics of the sample. Overall, the sample was quite diverse: 56.8% of the women were white, 62.7% of the women were single, 72.7% held a college degree, and 92.4% were full-time employed at one of the three medical centers at the time of the study. The remaining 7.6% of women were

hospital workers in less than a full-time employment situation. No significant demographic differences were observed between the groups, except for race χ^2=13.505, df=4, p<0.01. Planned contrasts of significant group effects revealed that women with first-degree relatives diagnosed with breast cancer at premenopausal age (HiFR group), as well as women with first-degree relatives diagnosed at postmenopausal age (LoFR) were less likely to be black compared to women without first-degree relatives with breast cancer (NoFR group) (p<0.01).

As shown in Table 2, group differences in medical history/health-related variables were found only for smoking on assessment day (χ^2=8.858, df=2, p<0.02). Planned contrasts revealed that women with first-degree relative diagnosed with breast cancer at postmenopausal age (LoFR group) were more likely to smoke on the assessment day compared to women without first-degree relatives with breast cancer (p<0.01).

Table 1: Socio-Demographic Variables of the Study Sample

Socio-demographic characteristics	NoFR [I] n=121 [II]	LoFR n=27	HiFR n=37
Mean age	34.9 ± 9.9	37.5±8.1	35.1±7.3
Ethnicity			
-Hispanic	13.3%	0%	13.5%
Race			
-White	51.7%	80.8%	56.8%
-Black	35.8% [a,b]	7.7% [b]	18.9% [a]
-Other	12.5%	11.5%	24.3%
Marital Status			
-currently married	33.1%	37.0%	51.4%
Education			
-some college or less	31.1%	14.8%	4.3%
-College degree	33.1%	40.7%	40.5%
-Graduate degree	35.3%	44.4	35.1%
Employment			
-full time	94.2%	88.9%	89.2%

[I] Mean ± standard deviation or percent (%) of total. NoFR Group (women without a first-degree relative with breast cancer); LoFR Group (women with at least one first-degree relative with breast cancer diagnosed after age 50); HiFR Group (women with at least one first-degree relative with breast cancer diagnosed before age 50).

[II] Number of subjects per group varies slightly across variables due to missing values for some factors.

Note. Logistic regressions with contrasts were conducted; columns with matching superscripts are significantly different ($p<0.05$).

Table 2: Medical History Variables of the Study Sample

Medical History	NoFR [I] n=121[II]	LoFR n=27	HiFR n=37
BMI	26.6 ± 6.4	24.8±5.6	24.3 ± 4.0
Children (≥1)	41.3%	33.3	45.9%
Birth control pills ever	68.6%	69.2%	80.6%
Cortisone regularly	7.6%	3.7%	2.8%
Smoked on assessment day	3.0% [a]	19.2% [a]	9.1%
Drank alcohol on assessment day	5.2%	0%	0%
Drank caffeine on assessment day	57.5%	77.8%	54.1%
Thoughts about BC during past 3 days	25.4%	23.1%	27.8%

[I] Mean ± standard deviation or percent (%) of total. NoFR Group (women without a first-degree relative with breast cancer); LoFR Group (women with at least one first-degree relative with breast cancer diagnosed after age 50); HiFR Group (women with at least one first-degree relative with breast cancer diagnosed before age 50).

[II] Number of subjects per group varies slightly across variables due to missing values for some factors.

Note. Logistic regressions with contrasts were conducted; columns with matching superscripts are significantly different ($p<0.05$).

To evaluate whether possible relationships between familial risk status and the cortisol excretion measurements could be confounded by the differences in socio-demographic and medical/health variables, separate univariate ANOVAs were computed with each cortisol assessment as the dependent variable and: (1) race as the between-subjects factor, and (2) smoking on assessment day as the between-subjects factor. None of the six ANOVAs revealed a significant test of between-subjects effects.

Familial Risk Group Differences: Cortisol Levels and Psychological Variables

Repeated measures ANOVA revealed a Group by Time interaction ($F[2,366]=3.177$, $p<0.05$) which led us to compute univariate follow-up ANOVAs at each time point. Work cortisol ($F[1,184]=4.774$, $p=0.03$), but not home ($F[1.184]=0.029$, $p=0.87$) or sleep cortisol ($F[1,184]=0.796$, $p=0.37$) differed between the groups, with planned contrasts indicating significant differences on work cortisol levels between HiFR and NoFR ($p=0.05$), but not between HiFR and LoFR or LoFR and NoFR (all $p>0.15$) (Figure 1, Table 3).

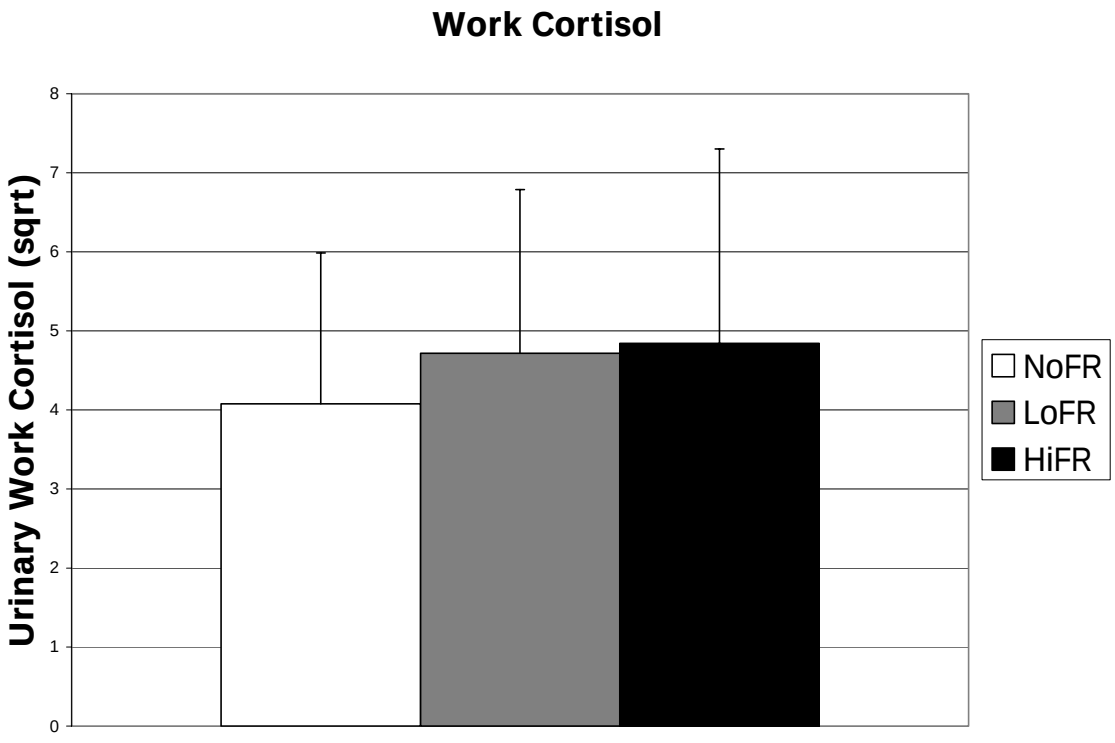

Figure 1. Mean work cortisol levels (sqrt) in women at no, low, and high familial risk (NoFR, LoFR, and HiFR, respectively)

Table 3: Descriptives for the Model Variables

Model Variables	NoFR n=121	LoFR n=27	HiFR n=37
Mean Work Cortisol (sqrt)	4.08±1.91 [a]	4.72±2.07	4.84±2.46 [a]
Intrusions ↑	55.4% [b]	59.3% [c]	89.2% [b,c]
Avoidance ↑	51.2% [d]	66.7%	73.0% [d]
BC Risk Perceptions ↑	38.0% [e,f]	63.0% [e]	83.8% [f]
Neg. Affect ↑	49.6%	61.5%	54.3%
Gen. Distress ↑	49.2%	55.6%	51.4%

Note. Logistic regressions with contrasts were conducted; columns with matching superscripts are significantly different ($p<0.05$).

Table 3 shows mean differences on work cortisol, as well as percentages for women with intrusions and avoidant behavior about breast cancer, as well as high values on breast cancer risk perceptions, negative affect, and general distress by familial risk group. HiFR women were more likely to have had at least one intrusion during the past week compared to the NoFR group (OR=6.655 [CI:2.250-19.687], Wald=11.734, $p<0.001$) and also compared to the LoFR group (OR=5.672 [CI:1.560-20.621], Wald=6.945, $p<0.009$); NoFR women and LoFR women did not differ (OR=1.172 [CI:0.502-2.735], Wald=0.135, $p=0.71$) (Figure 2). HiFR women were more likely to have had at least one avoidant behavior during the past week compared to the NoFR group (OR=2.569 [CI:1.145-5.776], Wald=5.235, $p=0.02$), but NoFR women and LoFR women did not differ (OR=1.903 [CI:0.793-4.570], Wald=2.073, $p=0.15$). HiFR women were also more likely to have had increased risk perceptions (OR=8.424 [CI:3.264-21.740], Wald=19.408, $p<0.001$); NoFR women and LoFR women also differed

(OR=2.772 [CI:1.169-6.570], Wald=5.366, p=0.02). Negative affect and general distress did not differ between the familial risk groups (all p-levels >0.3).

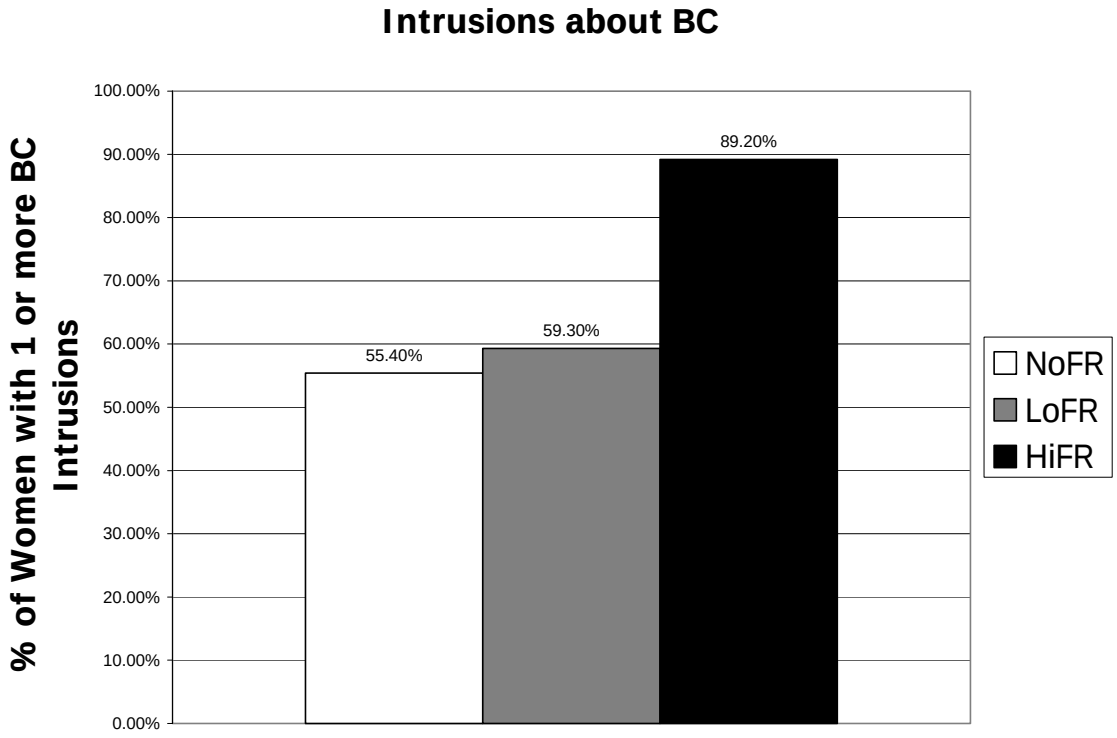

Figure 2. Percentage of Women with 1 or more breast cancer intrusions during the past week by familial risk group (no, low, high familial risk)

Possible Mediator Variables for Work-Stress Cortisol Responses: Intrusions, Avoidance, and/or Breast Cancer Risk Perceptions

To evaluate whether intrusions, avoidance, and/or breast cancer risk perceptions mediated the relationship between familial risk status and work cortisol levels, three separate univariate ANOVAs with the dichotomous variables of intrusions, avoidance, and perceived breast cancer risk, respectively, and work cortisol as the dependent

variable were conducted. Intrusions ($F[1,184]=9.240$, $p=0.003$), but not avoidance ($F[1,184]=1.961$, $p=0.16$) nor perceived breast cancer risk ($F[1,184]=0.724$, $p=0.40$) were related to work cortisol levels. It is of interest to note that neither negative affect nor general distress was related to cortisol responses. Univariate ANOVA with familial risk status (NoFR/LoFR/HiFR), and intrusions (dichotomized) as factors, and work cortisol as dependent variable indicated a significant contribution of intrusions to work cortisol ($F[1,184]=5.818$, $p<0.02$), with familial risk group experiencing a substantial reduction in its effect on work cortisol levels ($F[1,184]=2.569$, $p=0.12$). Familial risk group by intrusion group was not significant ($p=0.79$). However, analyses within the three groups indicated relations between intrusions and work cortisol among NoFR ($F[1,119]=6.532$, $p<0.02$), but not among LoFR ($F[1,25]=0.496$, $p=0.49$) and HiFR ($F[1,35]=0.038$, $p=0.43$).

Mood ratings during work and home: confirmation of the work environment as the stressful period of the day compared to the home environment

The design of this naturalistic experimental study approach considering work as the stressful period of the day is based on previous reports indicating that it is the work place that consistently elicits the strongest stress responses of the day (i.e.,(Brown et al., 2000; James et al., 1997; Kario et al., 2002). To confirm this notion in our sample, we analyzed mood ratings collected using a diary approach with assessments every 15 minutes in a subset of 116 participants. Subjects indicated whether they were anxious, angry, sad, or happy (all that apply) at any given time point. We first computed percentage of mood (anxious, angry, sad, and happy, respectively) recordings at

work/home (number of recordings mood at work/home x 100/total number of recordings at work/home). Paired Samples T-tests indicated a higher percentage of recordings for anxious during work compared to home ($p<0.001$). No differences were seen for angry, sad, and happy. Univariate analysis comparing mean percentage of anxious readings at work between NoFR (n=78), LoFR (n=17), and HiFR (n=21) indicated no differences between the groups ($F[2,115]=1.526$, $p=0.222$). Furthermore, we dichotomized the mood percentages into "not anxious/angry/sad/happy (respectively) at work/home" vs. "some anxious/angry/sad/happy (respectively) at work/home". A higher percentage of participants indicated that they were at least once anxious at work (48.7%) compared to 19.3% who indicated that they were at least once anxious at home. Chi-Square tests comparing lack of anxious readings at work with some anxious readings at work between familial risk groups indicated no differences between the groups on the dichotomized anxious variable ($\chi=0.748$, $p=0.688$) indicating that both groups experienced the work period as more stressful. There were no associations between percentage of anxious readings at work and work cortisol levels ($r=-0.007$, $p=0.937$) (see Appendix E).

Discussion

The present study investigated factors that could explain previously demonstrated increases in acute cortisol responses in women at familial risk for breast cancer. Consistent with study hypotheses, we found a significant gradient in work-stress cortisol levels across highest to lowest familial risk of breast cancer. While we found in the final

model a significant contribution of intrusions to work cortisol, with familial risk group experiencing a substantial reduction in its effect on work cortisol levels, follow-up analyses indicated that the relationship between intrusion and work cortisol was only apparent among the NoFR group. Furthermore, we found a significant group difference on intrusions between high and low familial risk, while work cortisol levels were not significantly different between high and low familial risk women. Hence, results provide conclusive evidence of intrusion effects, but leave uncertainty about mediation of group effects. In concordance with previous reports on psychological familial risk effects, perceived breast cancer risk and avoidance about breast cancer were increased in women with family histories of breast cancer, but these factors did not relate to work-stress cortisol responses. Negative affect and general distress did not differ between familial risk groups, nor did they predict cortisol responses.

The non-significant findings for a relationship between intrusions and work cortisol in the LoFR and HiFR group do not preclude a mediation effect of intrusions and could be explained by a lack of power to detect an effect, especially in the HiFR group, which consisted of four women without intrusions only. While the final regression model suggests a mediation effect of intrusions, the finding that mean work cortisol levels (Figure 1) were different between HiFR group and NoFR group, but not between HiFR and LoFR or LoFR and NoFR, whereas percentages of intrusions (Figure 2) were different between HiFR and NoFR, and also between HiFR and LoFR suggests the likelihood of additional mechanisms mediating the gradient increase in work cortisol levels from no familial risk, low familial risk, to high familial risk.

The finding of a significant gradient in work-stress cortisol levels across highest to lowest familial risk of breast cancer and the possibility for a mediation of this effect by intrusions about the disease among HiFR women is consistent with the possibility that an earlier age at diagnosis of the first-degree relative, in the majority of cases the mother, may have a different psychological impact on a woman due to several reasons: a) she is likely to be younger when her family is faced with the disease and the threat of losing a loved one, b) she is confronted with a more severe illness of her family member with more pathologic features, higher rate of local recurrence, and poorer prognosis (Zhou & Recht, 2004), and hence c) she is likely to have witnessed a more aggressive treatment with more severe adverse side effects. However, while breast cancer-specific distress was particularly pronounced in women with first-degree relatives diagnosed at premenopausal age, other indices of general distress and negative affect which have previously been reported to differ between family history groups in some studies (Baider et al., 1999; Valdimarsdottir et al., 1995) but not others (Butow et al., 2004; Coyne, Benazon, Gaba, Calzone, & Weber, 2000) were not confirmed in the present analyses.

The finding that intrusions about breast cancer were related to work-stress cortisol levels, while risk perceptions, avoidance about breast cancer, negative affect, and general distress were not related to cortisol in this sample of healthy women with and without histories of breast cancer, is consistent with a growing literature suggesting that it is the identification of stressor specific cognitions and emotions that is more predictive of biological stress responses, as compared to more general measures of distress and negative affect (Lutgendorf et al., 2001; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005).

While a number of reports have demonstrated a relationship between intrusions about a specific stressor and HPA axis measures (Antoni et al., 1990; Baum et al., 1993; Lutgendorf et al., 2001), to our knowledge, this is the first report demonstrating a relationship between intrusions about a specific stressor (breast cancer) and cortisol responses to unrelated acute stress (work). These data support Gump and Matthews' (Gump et al., 1998) theorizing that repeatedly coping with an ongoing stressor (here the threat of breast cancer) would contribute to chronic or repeated arousal, as well as priming for a stronger response to unrelated acute stressors. Considerable research in animals and humans provides compelling evidence that acute stress responses are increased in the presence of background stressors (Bhatnagar, Mitchell, Betito, Boksa, & Meaney, 1995; Gump et al., 1999), albeit with fewer and more variable results for HPA axis measures (Cacioppo et al., 2000; Matthews, Gump, & Owens, 2001). While the mechanisms responsible for the effects of background stress on acute stress responses are unresolved, the present data suggest that neither awareness about breast cancer risk, nor avoidance of breast cancer, but rather intrusions about the disease are the critical link to increased cortisol levels in response to unrelated acute stress. It should be noted, however, that these findings do not preclude the possibility that other factors not examined here (i.e., genotype (Wust et al., 2004)) may also contribute to the familial risk group differences in acute cortisol responses.

The present study has limitations that must be recognized. First, participation in a breast cancer study may have elicited positive responses for intrusions on the IES during the week before coming for the study appointment, particularly for women at familial risk for the disease. Hence, generalizability of the study results regarding the duration of

effects may have to be interpreted with caution. However, 73.2% women had negative responses to all three items "Have you had thoughts about breast cancer today/yesterday/2 days ago;" there was no difference between the familial risk groups on this measure, nor any increase in the reports of thoughts about breast cancer as the assessment day grew nearer (see Appendix C). Second, the present study did not include self-report assessments of perceived stress at work. However, considerable evidence suggests that it is the work place that consistently elicits the strongest stress responses of the day (i.e., Brown et al., 2000; James et al., 1997; Kario et al., 2002), with support from a related study drawing from the same population of working women sampled here indicating that self-reported anxiety levels at work were higher than in the home environment (see Appendix E).

In conclusion, the present study indicates conclusive evidence of intrusion effects, but leaves uncertainty about mediation of group effects. A larger sample size for the HiFR group is needed to increase variance on intrusion scores to obtain conclusive results for the hypothesis that intrusions about breast cancer provide one psychological mechanism linking familial risk status with heightened responses to acute stressors. To our knowledge, this is the first study to show a gradient increase in cortisol levels during work with increasing familial risk of breast cancer. This is also the first study to link cortisol obtained from a naturalistic setting of three different stressor environments to stress by intrusions about breast cancer in women at different levels of familial breast cancer risk. Further studies are now warranted with larger sample sizes to replicate intrusion effects on work cortisol levels and the possibility for mediation of gradient cortisol increases from no familial risk, low familial risk, to high familial risk groups by

intrusive thoughts about breast cancer. Future studies should also explore predictors of intrusions about breast cancer, particularly in women at high familial risk for the disease. Additionally, the possibility that intrusions may generally serve as a psychological mediator of heightened reactivity associated with other background stressors deserves further research.

Lower levels of overall adiposity but larger adjusted waist circumferences in healthy premenopausal women at familial risk for breast cancer[1]

Abstract

Objective: To examine overall adiposity and central obesity, two well-established risk factors for breast cancer, in healthy premenopausal women with at least one first-degree relative with breast cancer either diagnosed at premenopausal age or postmenopausal age compared to healthy women without a first-degree relative with the disease. *Methods*: 75 healthy premenopausal women with at least one first-degree relative diagnosed at premenopausal age (HiFR, n=41) or at postmenopausal age (LoFR, n=34), and 137 women without a first-degree relative with the disease (NoFR) participated in this study. Measures of overall adiposity (weight and height), intra-abdominal fat (waist circumference) and abdominal subcutaneous fat (waist skinfold) were collected in addition to sociodemographics and medical history information. *Results:* Logistic regression with familial risk status as the outcome and race, body mass index (BMI), and waist circumference as predictors demonstrated significant independent contributions in the opposite direction of BMI and waist circumference for being in the HiFR group compared to the NoFR group (OR=0.21; 95% CI=0.08-0.53; p=0.001 and OR=4.23; 95% CI=1.80-9.91; p=0.001, respectively). Identical analysis with waist skinfold instead of

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waist circumference did not yield significant regression parameters for BMI or waist skinfold. Consistent with the logistic regression results, univariate ANCOVA indicated lowest BMIs (adjusted Mean=24.148) in HiFR compared to LoFR (adjusted Mean=25.767; p=0.018) and NoFR (adjusted Mean=26.193; p<0.001). In contrast, highest adjusted waist circumferences were found for HiFR (adjusted Mean=81.745) compared to LoFR (adjusted Mean=77.463; p=0.003) and NoFR (adjusted Mean=77.824; p<0.001). *Conclusions*: These results support the possibility that familial aggregation of low overall adiposity but relatively high levels of central adiposity at premenopausal age, with its associated metabolic consequences, may contribute to familial risk of breast cancer.

Introduction

The increased risk of developing breast cancer among women with family histories of the disease has long been recognized (Pharoah et al., 2002). Healthy women with family histories that include even a single first-degree relative with breast cancer have a significantly elevated risk of developing the disease themselves (i.e., relative risk 1.8) compared to women without breast cancer in close relatives (Collaborative Group, 2001). The risks increase further if other characteristics consistent with inherited susceptibility are present in the family, such as multiple affected relatives and younger ages at diagnosis (i.e., before age 50) (Collaborative Group, 2001) (Hopper, 2001a). Risk estimates by family history are particularly high for premenopausal women suggesting a stronger contribution of genetics to premenopausal breast cancer (Pharoah, Day, Duffy, Easton, & Ponder, 1997). In 1990 when linkage analyses of large kindreds with multiple affected family members over several generations identified germ-line mutations in BRCA1 (Hall et al., 1990), followed by the identification of BRCA2 in 1994 (Wooster et al., 1994), it was widely believed that these autosomal dominant susceptibility genes would account almost entirely for familial risk (Hopper, 2001b). Subsequent population based studies however, have indicated that after accounting for the contribution of mutations on these two known breast cancer susceptibility genes to risk, family history is still associated with a significant increase in lifetime risk of breast cancer (Claus, Schildkraut, Iversen, Jr., Berry, & Parmigiani, 1998; Kaufman & Struewing, 1999). Indeed, the contribution of mutations in BRCA1 and BRCA2 to familial risk more generally has recently been estimated to be approximately 15% (Pharoah et al., 2002).

The modest contribution of BRCA mutations to the increased risk of breast cancer associated with having a first-degree relative with breast cancer has awakened new interest in exploring additional factors that may contribute to familial risk and recognition that other genetic models including high-penetrance recessive genes and polygenic effects may contribute to familial risk (Pharoah et al., 2002; Pharoah et al., 2002). For example, Pharoah and colleagues (Pharoah et al., 2002) have proposed a polygenic model in which many genes with only a weak contribution individually may additively or multiplicatively contribute to increased risk of breast cancer among women with family histories of the disease.

One biologically plausible factor that could contribute to familial risk, but has gotten little research attention in that regard, is overall adiposity. A pooled analysis of seven prospective cohort studies, together comprising 337,819 women, found inverse associations with overall adiposity measured by body mass index (BMI) for premenopausal breast cancer in each study. In contrast, in postmenopausal women, BMI was positively associated with breast cancer in all studies but one, in which no association was found. The pooled relative risk for a BMI increment of 4 kg/m^2 was 0.89 in premenopausal women, and 1.07 in postmenopausal women (van den Brandt et al., 2000). Among the hypothesized biologic mechanisms to explain how overall adiposity might protect against breast cancer risk in premenopausal women, but enhance breast cancer risk in postmenopausal women are decreased estradiol concentrations with increasing BMI in premenopausal women, but increased estradiol concentrations with increasing BMI in postmenopausal women (Verkasalo, Thomas, Appleby, Davey, & Key, 2001). Relatively high estradiol concentrations are associated with an increase in

breast cancer risk in premenopausal and postmenopausal women (Key, 1999; Yu et al., 2003).

Another biologically plausible factor with little research attention among women with a family history of breast cancer is central obesity. Although not without exception (Petrek, Peters, Cirrincione, Rhodes, & Bajorunas, 1993), recent reviews (Friedenreich, 2001; Harvie, Hooper, & Howell, 2003) and a meta-analysis by Connolly and colleagues (Connolly et al., 2002) have concluded that there is compelling evidence that central obesity, operationally defined by waist circumference or waist-to-hip ratio, is an independent risk factor for breast cancer after controlling for overall adiposity. For example, Mannisto et al. (Mannisto et al., 1996) found waist-to-hip ratio to be a significant risk factor for breast cancer in both premenopausal and postmenopausal women, controlling for other well known risk factors (e. g., age at menarche, age at first full-term pregnancy, age at menopause). Among the hypothesized biologic mechanisms to explain how central obesity might influence breast cancer risk are positive correlations with levels of endogenous estrogen, insulin, and growth factors that have been shown to promote breast cancer cells (Hankinson et al., 1998; Pollak, 2000; Toniolo, 1997). While central obesity can be a result of lifestyle habits, such as physical activity, or dietary intake, several twin and family studies have shown that there is a strong genetic influence with estimates that up to 51% of the phenotypic variance that is explained after adjustments for the effects of age and age plus total fat mass (Bouchard et al., 1996; Olson, Atwood, Grabrick, Vachon, & Sellers, 2001; Perusse et al., 1996; Samaras & Campbell, 1997).

Based on the literature highlighted above, the present study was designed to provide a first critical test of the possibility that the inheritance of a pattern of a low BMI but a relatively high waist circumference, previously shown to be risk factors for premenopausal breast cancer, may contribute to familial risk of this disease, and particularly high risk estimates for premenopausal women with first-degree relatives diagnosed at premenopausal age. Specifically, we tested the hypothesis that healthy premenopausal women with at least one first-degree relative with breast cancer diagnosed at premenopausal age have lower levels of overall adiposity, measured by BMI, and higher levels of central obesity, measured by waist circumference adjusted for BMI, than women with a first-degree relative diagnosed at postmenopausal age and women without a first-degree family member with breast cancer, controlling for other factors that might confound this relationship.

To our knowledge, no study has examined overall adiposity levels in relationship to family history of breast cancer, and only one previous study, conducted over a decade ago, has examined variation in body fat distribution, and reported significantly larger waist-to-hip ratios in 56 healthy women with family histories of breast cancer compared to 56 healthy women without such histories controlling for overall adiposity (Schapira, Kumar, & Lyman, 1993). The present study expands upon this previous research by investigating the unique variance of both overall adiposity and central obesity; by specifically focusing on premenopausal women because overall adiposity as a risk factor for breast cancer is reversed by menopausal status; by considering potentially confounding characteristics that might be related to overall adiposity, central obesity, and breast cancer risk (i.e., parity); by examining surrogate measures of intra-abdominal fat

levels (waist circumference adjusted for BMI) and abdominal subcutaneous fat levels (waist skinfold adjusted for BMI); and by exploring overall adiposity and central obesity in women with different familial risk levels (diagnosis <50 years vs. ≥ 50).

Materials and Methods

Description of study population

A total of 212 healthy women between the ages of 25 and 49 (premenopausal age) participated in this study, a subset of women of all ages recruited for a larger study on different family histories of breast cancer. Participants were recruited through advertisements for a study of healthy women with different family histories of breast cancer at three major medical centers in New York City. Over 90% of the women who responded to the ads enrolled in the study. Participants of this study were seen between March of 1998 and October of 2000. The following exclusion criteria for participation in the study were applied: 1) not English speaking, 2) a history of HIV, cancer or abnormal breast exams (including abnormal breast biopsy or abnormal mammogram), 3) medication use other than birth control pills, and 4) participation in any other research study that could potentially affect study variables. The study was conducted under institutional review board approval and all participants signed informed consent. Based on their self-reported family histories of cancer women were classified the following way: as a way to explore age at diagnosis of the first-degree relative as a well-known marker of transmission of breast cancer, we divided the women with first-degree relatives

with breast cancer into women with at least one first-degree family member diagnosed before age 50 (premenopausal age) (HiFR) (n=41), and women with all first-degree family members diagnosed at age 50 or older (postmenopausal age) (LoFR) (n=33). One woman could not be categorized due to missing relative information and dropped out of the analysis.

Procedures and Measures

On the day of the study assessment, participants completed informed consent and self-administered questionnaires eliciting detailed information on demographic factors, family histories of cancer, reproductive and menstrual history, hormone use, smoking habits, and alcohol consumption. Anthropometric measurements, including height, weight, waist circumference, hip circumference, and waist skinfold were collected by trained research personnel using well-established standardized methods (Weiner & Lourie, 1981). All measurements were taken while the participant was standing in an erect position. Height was measured using a stadiometer; weight with a clinical scale. Waist circumference was measured at the umbilicus. Body mass index (BMI) was calculated (weight/height^2). Previous independent research has shown that waist circumference has a high correlation with intra-abdominal fat calculated from computed tomography (Ashwell, Cole, & Dixon, 1996), and that BMI and waist circumference independently contribute to the prediction of visceral fat (Janssen, Heymsfield, Allison, Kotler, & Ross, 2002). Waist skinfold measurements were based on the mean of triplicate readings with a Lange caliper holding the skin in a vertical position and pinching it three times. Waist skinfold was measured two inches to the left of the

umbilicus. Skinfold measurements are used to estimate subcutaneous fat mass (Ashwell, 1994), and have been shown to only correlate to subcutaneous abdominal fat quantified by computer tomography (Ribeiro-Filho, Faria, Azjen, Zanella, & Ferreira, 2003).

Statistical analyses

First, univariate analyses were performed using Pearson chi-square for categorical variables and analysis of variance (ANOVA) for continuous variables to investigate differences between groups on variables suspected to be associated with BMI and waist circumference. To investigate confounding (association with risk factor and outcome), Spearman correlations were conducted between BMI and waist circumference and variables significantly different between the groups.

Second, logistic regression was applied to assess prediction of membership in one of three categories of outcome (NoFR, HiFR, and LoFR). Continuous predictor variables were transformed into z-scores for this analysis to avoid problems of multicollinearity in including interaction terms of predictors. In step one identified confounders entered the model as covariates and BMI and waist circumference as the main predictor variables. A separate model was calculated with the addition of BMI by waist circumference to evaluate a possible contribution of the interaction term. The same analysis was performed with waist skinfold instead of waist circumference. The log-likelihood test was used to determine if the predictors, as a group, contribute to prediction of the outcome. Odds ratios (OR) and their confidence intervals along with the Wald statistic were used to determine significant contributions of the corresponding regression parameter.

Third, analyses of covariance (ANCOVA) were used to investigate mean group differences in BMI, waist circumference, and waist skinfold by NoFR/LoFR/HiFR status adjusting for in step one identified confounders.

All analyses were performed using SPSS for Windows Version 11.5.

Results

Possible confounding differences between the groups on demographic characteristics were statistically evaluated. As shown in Table 1, significant differences were found for race only, and as a trend for marital status. Overall, the sample was composed of predominantly white (56.5%), single (60.8%) women, who were highly educated (72.3% college degree or above), and holding a full-time employment at the time of study assessment (91.5%).

Possible confounding differences between the groups on lifestyle and medical history variables that may influence overall adiposity and abdominal fat were statistically evaluated. As shown in Table 2, no such differences were found.

To assess whether the relationship between familial risk status and the primary independent variables, BMI and waist circumference, could be confounded by those socio-demographic variables that differed between the groups, correlations with BMI and waist circumference were computed (Table 3) (Hosmer and Lemeshow, p. 63). Because race showed associations with both familial risk status and BMI and waist

circumference, it was identified as a confounder and entered the subsequent analyses as a covariate.

Table 1: Socio-demographic characteristics of the study sample by familial risk group.[a]

Socio-demographic characteristics	**NoFR n=137** [b]	**LoFR n=34**	**HiFR n=41**	**Statistical test value**	**p-value**
Mean age	34.7 ± 0.6	36.9±1.3	34.8±0.5	F(2,211)=1.293	p=0.277
Ethnicity -Hispanic	11.9%	6.1%	7.3%	χ^2=1.405, df=2	p=0.495
Race -White	51.9%	75.8%	56.1%		p=0.003*
-Black	34.8%	18.2%	14.6%	χ^2=16.030,	
-Other	13.3%	6.1%	5.7%	df=4	
Marital Status -currently married	33.6%	50.0%	48.8%	χ^2=5.063, df=2	p=0.080
Education -some college or less	33.3%	17.6%	17.1%	χ^2=6.617,	p=0.158
-College degree	35.6%	41.2%	39.0%	df=4	
-Graduate degree	31.1%	41.2%	43.9%		
Employment -full time	91.2%	91.2%	92.7%	χ^2=0.090, df=2	p=0.956

[a] Mean ± standard error or percent (%) of total. NoFR Women without a first-degree family member with breast cancer; LoFR Women with at least one first-degree family member with breast cancer diagnosed at postmenopausal age; HiFR Women with at least one first-degree family member with breast cancer diagnosed at premenopausal age.

[b] Number of subjects varies by up to 7 subjects in both groups across variables due to missing values.

Table 2: Medical History Variables of the study sample [a]

Medical History	**NoFR n=137** [b]	**LoFR n=34**	**HiFR n=41**	**Statistical test value**	**p-value**
Smoking ever	34.8%	41.2%	37.5%	χ^2=0.503, df=2	p=0.778
Smoking (past month)	12.7%	21.2%	15.4%	χ^2=1.573, df=2	p=0.455
Alcohol consumption (past month)	66.4%	67.6%	80.0%	χ^2=2.716, df=2	p=0.257
Mean age at menarche	12.5 $\pm$ 0.1	12.5$\pm$0.2	12.6$\pm$0.2	F(2,198)=0.76	p=0.927
Number of children	0.9	0.6	0.7	F(2,211)=1.021	p=0.362
Birth control pills ever	72.3%	66.7%	82.5%	χ^2=2.549, df=2	p=0.280
HRT ever	1.5%	0%	4.9%	χ^2=2.677, df=2	p=0.262
Cortisone regularly	8.1%	5.9%	2.5%	χ^2=1.618, df=2	p=0.445

[a] Mean $\pm$ standard error or percent (%) of total.
[b] Number of subjects varies by up to 7 subjects in both groups across variables due to missing values.

Table 3: Spearman correlations between the main outcome variables and socio-demographic variables with group differences (p<0.10)

	BMI	**Waist circumference**
Race	r=.153* p=.027	r=.195* p=.005
Marital status	r=.064 p=.357	r=.026 p=.703

Logistic regression analysis was performed to assess prediction of membership in one of three categories of outcome (NoFR/LoFR/HiFR) on the basis of race, BMI, and waist circumference. Goodness-of-fit statistics with all predictors in the model showed an excellent fit with p=.995 by the Deviance criterion and with p=.575 by the Pearson criterion. Parameter estimates comparing HiFR women with NoFR women indicated significant contributions of BMI, waist circumference, and race to the prediction. HiFR

women were less likely to have increased BMIs (OR:0.207, 95% CI:0.081-0.531), but more likely to have increased adjusted waist circumferences (OR:4.226, 95% CI:1.802-9.907), adjusting for race. The Likelihood Ratio Tests further indicated that the three predictors reliably distinguished among outcomes: BMI (χ^2=13.753, p=.001), waist circumference (χ^2=14.816, p=.001), and race (χ^2=12.030, p=.017).

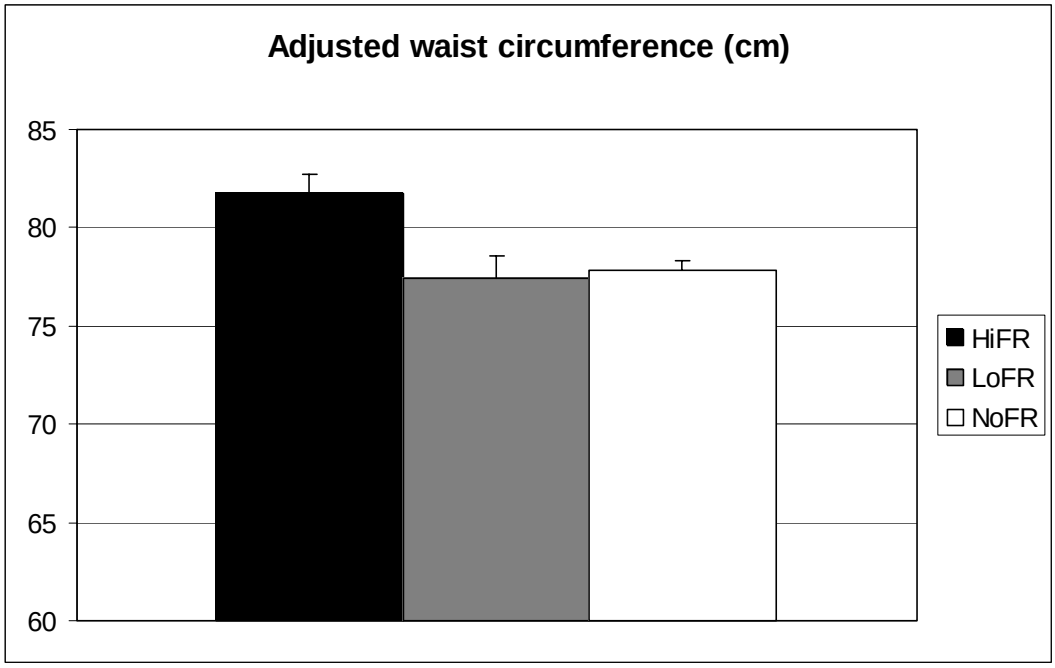
Table 4: Logistic regression predicting familial risk (NoFR/LoFR/HiFR) of breast cancer

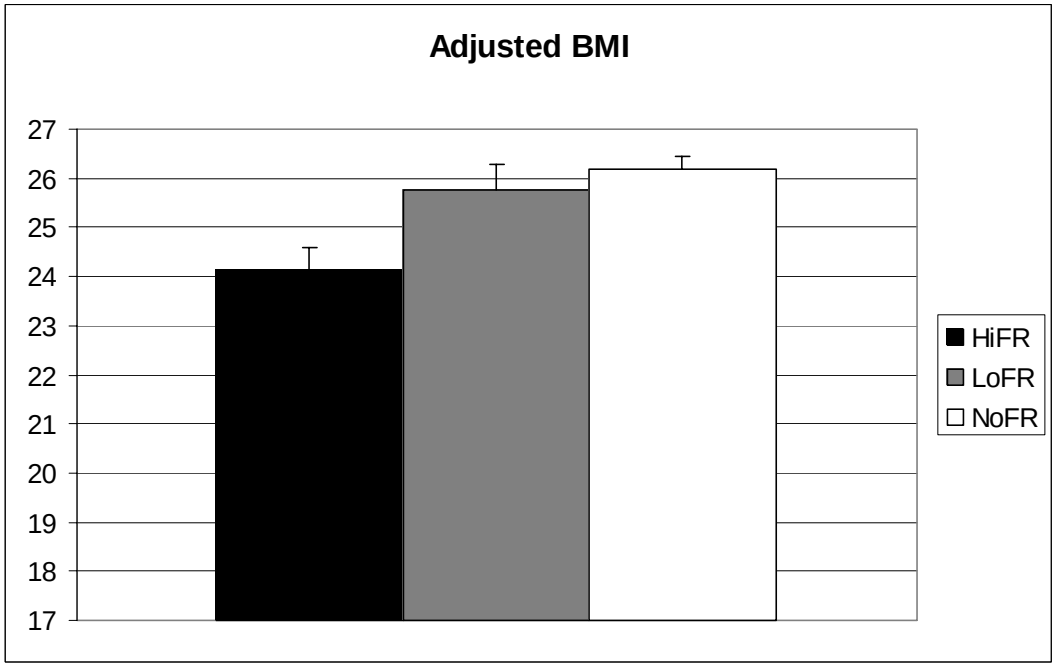
Logistic regression model[a] (p<0.001, r^2=.184[b]):	B	S.E.	Wald	df	Sig.	Exp (B)	C.I. for Exp (B)
LoFR:							
Race							
-white vs. other	1.175	0.784	2.247	1	0.134	3.238	0.697-15.050
-black vs. other	.381	0.875	0.190	1	0.663	1.464	0.264-8.131
BMI	-0.298	0.437	0.465	1	0.495	0.742	0.315-1.747
Waist Circumference	-0.184	0.435	0.179	1	0.672	0.832	0.355-1.950
HiFR:							
Race							
-white vs. other	-0.634	0.472	1.804	1	0.179	0.530	0.210-1.338
-black vs. other	-1.533	0.605	6.413	1	0.011	0.216	0.066-0.707
BMI	-1.576	0.481	10.742	1	0.001	0.207	0.081-0.531
Waist Circumference	1.441	0.435	10.990	1	0.001	4.226	1.802-9.907

[a]all predictor variables are transformed standardized z-scores
[b]Nagelkerke R Square

Univariate ANCOVAs with planned contrasts were conducted to investigate levels of BMI (adjusted for race and waist circumference) and waist circumference (adjusted for race and BMI). Consistent with the results from the logistic regression analysis, univariate ANCOVAs indicated a main effect of group on BMI (F[4,209]=8.077, p<0.001, eta^2=0.073) and waist circumference (F[4,209]=6.962, p=0.001, eta^2=0.064), adjusting for race. Planned comparisons indicated that HiFR

women have the lowest BMI (adjusted Mean=24.148) compared to LoFR women (adjusted Mean=25.767, p=0.018) and NoFR women (adjusted Mean=26.193, p<0.001); NoFR and LoFR did not differ (p=0.451). For waist circumference, planned comparisons indicated that HiFR women have the highest waist circumferences (adjusted Mean=81.745) compared to LoFR women (adjusted Mean=77.463, p=0.003) and NoFR women (adjusted Mean=77.824, p<0.001); NoFR and LoFR did not differ (p=0.766). Consistent with the hypothesized specificity of this group difference for intra-abdominal fat, waist skinfold was not different between the groups (F[4,208]=0.402, p=0.669) (see Figure 1).


Adjusted waist circumference (cm)
85
80
75
70
65
60
HiFR
LoFR
NoFR


Adjusted BMI
27
26
25
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22
21
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17
HiFR
LoFR
NoFR

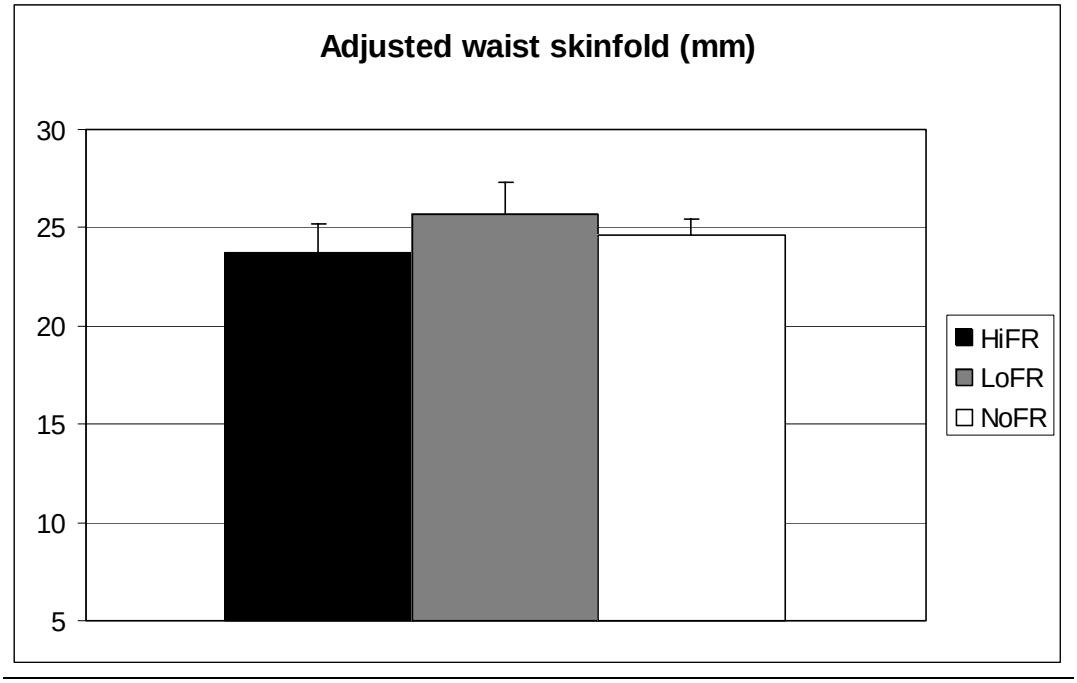

Figure 1: Group differences for waist circumference (adjusted for race and BMI), BMI (adjusted for race and waist circumference), but not for waist skinfold (adjusted for race and BMI).

Note: a) Error bars indicate standard error of adjusted means

b) Minimum values represent the lowest data point in our sample (rounded to nearest scaling unit on the abscissa)

Discussion

The present study was designed to provide a first critical test of the hypothesis that premenopausal women at high familial risk for breast cancer (at least one first-degree relative diagnosed at premenopausal age) have lower levels of overall adiposity but higher levels of intra-abdominal fat than women at low familial risk for the disease (a first-degree relative diagnosed at postmenopausal age) or women without first-degree relatives with the disease, controlling for overall levels of fat. Three anthropometric variables reflecting a) overall adiposity (BMI), b) intra-abdominal fat (waist circumference controlling for overall adiposity), and c) subcutaneous abdominal fat (waist skinfold control for overall adiposity) were assessed in our sample of 212 women. Consistent with our hypotheses, we found lower levels of overall adiposity but higher levels of intra-abdominal fat among women at high familial risk for breast cancer compared to women at low familial risk and no familial risk for the disease. These findings are consistent with data published a decade ago by Schapira et al. (Schapira et al., 1993), which to our knowledge, is the only other published result supporting a relationship between intra-abdominal fat levels and familial risk of breast cancer.

In addition to measures of intra-abdominal fat, also assessed in the present study were measures of subcutaneous abdominal fat. In contrast to Schapira's findings (Schapira et al., 1993), the results of the present study did not reveal a group difference on subcutaneous fat measures. This incongruence between the two studies could stem from differences in the study populations. While we investigated first-degree relatives of breast cancer patients diagnosed a mean of 6 years prior to study entry, participants in the

prior study are described as first-degree relatives of breast cancer patients diagnosed within a year, most of whom were probably still in treatment. Having a relatively recently diagnosed breast cancer patient in the family may be sufficiently stressful to result in changes of eating behavior, which in turn could result in a preferential gain of subcutaneous adipose tissue. This possibility would be entirely consonant with a considerable literature documenting stress-induced eating and weight gain (Epel, Lapidus, McEwen, & Brownell, 2001) and accumulation of subcutaneous fat at the expense of intra-abdominal fat in acute weight gain (van der, Leenen, Seidell, Deurenberg, & Hautvast, 1993; Zamboni et al., 1997).

The present results provide the second indication in the literature that intra-abdominal fat is greater in women at familial risk for breast cancer compared to women at population risk for the disease. It expands on the earlier finding by a) indicating that it is women with first-degree relatives diagnosed at premenopausal age, which is consistent with a high familial risk, who have higher intra-abdominal fat levels, and b) also demonstrating lower overall adiposity. The increased lifetime risk of premenopausal and postmenopausal breast cancer among women with a family history of the disease is well established (Collaborative Group, 2001), and only partially explained by currently identified breast cancer susceptibility genes (Easton, 1999; Ponder, 2001). Intra-abdominal fat is a risk factor for pre- and postmenopausal breast cancer, with a recent systematic review suggesting amongst pre-menopausal women, abdominal fat adjusted for overall adiposity may be specifically associated with an increased risk of breast cancer, because while adjustment for BMI abolished the relationship between waist or WHR and risk of post-menopausal breast cancer, it introduced such a relationship

amongst pre-menopausal women (Harvie et al., 2003). Higher levels of intra-abdominal fat in women at familial risk for breast cancer are potentially contributing to familial risk of the disease.

Intra-abdominal and subcutaneous abdominal fat are functionally and morphologically different. Compared to subcutaneous abdominal fat cells, intra-abdominal fat cells are more metabolically active with: a) increased androgen production and estrogen synthesis through aromatase activity, b) higher fatty acid turnover and lipolysis, c) lower responsiveness to the antilipolytic effect of insulin, d) a higher density of glucocorticoid receptors, and e) more 11β-hydroxysteroid dehydrogenase-1 and interleukin-6 (IL-6) production, among others (Wajchenberg, Giannella-Neto, Da Silva, & Santos, 2002). The metabolic characteristics of intra-abdominal fat contribute to higher endogenous estrogen, free fatty acid, cortisol, and insulin concentrations, insulin resistance, and lowered sex hormone-binding globulin (SHBG) levels, effects, which may be related to increased risk of breast cancer (10,11,12). For example, estrogens are well known as initiators of breast cancer (by increasing breast cell proliferation and genetic instability perhaps by inducing free radical-mediated DNA damage and mutations), or proliferator of breast cancer (by stimulating the growth of existing malignant cells, (Stoll, 2002; Hilakivi-Clarke, 2000)

The present study is consistent with genetic influences on intra-abdominal fat, but is also open to systematic environmental and behavioral differences between women at high familial risk for breast cancer and women at low or no familial risk for the disease. While we are unable to tease apart the underlying mechanism for a higher incidence of intra-abdominal fat in women at high familial risk for breast cancer, we know, that our

finding cannot be attributed to the following variables that have been demonstrated to have an influence on intra-abdominal fat: exogenous hormones (oral contraceptive use, hormone replacement therapy, and cortisone, (Bujalska, Kumar, & Stewart, 1997; Mattiasson, Rendell, Tornquist, Jeppsson, & Hulthen, 2002; Rebuffe-Scrive, 1988; Tchernof, Poehlman, & Despres, 2000), cigarette smoking (Daniel, Martin, & Faiman, 1992; Shimokata, Muller, & Andres, 1989), alcohol intake (Laws, Terry, & Barrett-Connor, 1990), age at menarche (Kirchengast, Gruber, Sator, & Huber, 1998), parity (den, I, Seidell, van Noord, Baanders-van Halewijn, & Ouwehand, 1990), and menopausal status (Svendsen, Hassager, & Christiansen, 1995). None of these variables accounted for the group differences in our analyses.

In addition to being significant, it is important to note that the effect size for the difference in intra-abdominal fat between HiFR women and NoFR women are comparable to other well-established risk factors of intra-abdominal fat. When we compare the effect sizes of the present study to studies demonstrating a significant influence of cigarette smoking and parity on intra-abdominal fat deposition, the difference between our groups is equal to premenopausal women with 0 vs. 6 children, or premenopausal women who currently smoke 0 to 10 cigarettes a day vs. more than 20 cigarettes a day (with BMIs between 25 and 30), based on a cross-sectional study in 11 825 Dutch women participating in the DOM- Project (den, I et al., 1990).

The data for the present research was based on a sample of 212 healthy women between the ages of 25 and 49. Although computed tomography or magnetic resonance imaging are the gold standards for assessing the two fat compartments, we used simple anthropometric measurements (BMI and waist circumference for overall adiposity and

intra-abdominal fat measures, and waist skinfold subcutaneous abdominal fat measures) relying on previous research that has demonstrated that waist circumference has correlations with intra-abdominal fat (Ashwell et al., 1996), and also relying on a recent systematic review indicating amongst pre-menopausal women, central (not general) adiposity may be specifically associated with an increased risk of breast cancer, using adjustment for BMI, which abolished the relationship between waist or WHR and risk of post-menopausal breast cancer, but introduced such a relationship amongst pre-menopausal women (Harvie et al., 2003).

In summary, based on our results, we propose that the familial aggregation of intra-abdominal fat, with its associated metabolic consequences, may contribute to the unexplained familial aggregation of breast cancer. Studies using computed tomography or magnetic resonance imaging are now warranted to confirm this initial indication of higher levels of intra-abdominal fat in healthy women at high familial risk of breast cancer compared to women at normal risk. Studies investigating the genesis of intra-abdominal fat in healthy women at high familial risk are needed to develop appropriate intervention strategies to reduce the risk of breast cancer in families with a history of the disease.

Exploratory analysis: Cortisol in relation to central fat in premenopausal women at different levels of familial risk for breast cancer[1]

Abstract

Objective: The goal of the present analysis was to explore associations between central adiposity and urinary cortisol levels during work in premenopausal women at different levels of familial risk for breast cancer (no, low, and high familial risk). *Methods:* Measures of weight, height, and waist circumference were collected by trained research assistants. Urinary cortisol excretion rates were assessed with timed sample collections at work (i.e., 11AM to 3PM), home (i.e., 6PM to 10PM), and during sleep (i.e., 10PM to 6AM). We computed linear regression models indicating zero-order correlations and part correlations for each variable in the model (waist circumference, BMI and familial risk status) following a literature indicating that waist circumference is a predictor for premenopausal breast cancer after controlling for BMI. We also analyzed delta cortisol values (work-nadir (home or sleep)). *Results:* Part correlations indicated a unique contribution of 1% (non significant) of waist circumference (adjusted for BMI and familial risk groups) to work cortisol levels with lower waist circumference being associated with higher work cortisol levels (part r=-0.102); familial risk status exerted highest contributions to work cortisol levels (part r=0.206 and part r=0.124 for high and

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low risk, respectively). Multivariate analysis indicated a significant main effect of familial risk status on work cortisol after adjusting for abdominal fat with planned contrasts indicating a significant difference between HiFR and NoFR ($p=0.009$). Future studies should investigate the underlying mechanisms of the apparently independent concurrence of increased cortisol reactivity and increased levels of central adiposity (controlled for general adiposity) in women at high familial risk for breast cancer.

Introduction

Associations between cortisol and central adiposity have been shown repeatedly in the last decade (Bjorntorp, 2001) (for review). A number of studies have demonstrated elevated cortisol levels in response to stimulation of the HPA axis in the presence of central adiposity. Such stimulations include food intake (Rosmond, Holm, & Bjorntorp, 2000), perceived stress (Rosmond & Bjorntorp, 1998), and direct stimulation with corticotropin-releasing hormone or adrenocorticotropin (Kopelman et al., 1988; Marin et al., 1992; Pasquali et al., 1993). Hypercortisolism in the presence of central adiposity has been explained by a hypersensitive HPA axis, an explanation supported by results indicating a decrease in the inhibition of cortisol secretion by dexamethasone with elevated waist-to-hip ratios in men (Ljung, Andersson, Bengtsson, Bjorntorp, & Marin, 1996). This decrease in inhibition may indicate a blunted feedback control by central glucocorticoid receptors, possibly a functional consequence of an elevated HPA axis activity. While the majority of studies do not control for overall obesity which creates difficulties in interpreting the results of a hyperactive HPA axis as specifically linked to central adiposity independent of overall adiposity, there is indication, that central adiposity is related to greater psychological vulnerability to stress and cortisol reactivity among lean women also (Epel et al., 2000).

Other researchers point out the important role of glucocorticoids to regulate adipose tissue differentiation, function, and distribution (Bujalska, Kumar, & Stewart, 1997; Gaillard, Wabitsch, Pipy, & Negrel, 1991; Hauner, Schmid, & Pfeiffer, 1987). Three decades ago, Naeser (Naeser, 1973) already demonstrated, that adrenalectomy in

the ob/ob mouse prevents the development of obesity. In excess, glucocorticoids can clearly cause central obesity, as is exemplified in patients with Cushing's syndrome or in patients receiving corticoisteroid therapy with a reduction of fat mass with removal of cortisol excess (Raff & Findling, 2003). The activity of cortisol leading to accumulation of fat in the central area of the body, specifically, involves several processes: cortisol activates lipoprotein lipase, the gate-keeper of lipid accumulation in adipocytes, and cortisol in the presence of insulin inhibits the lipid mobilizing system. Both events are mediated by glucocorticoid receptors, the binding site for cortisol, which show a particularly high density in central fat compared to other fat depots. Given these effects of cortisol on lipid mobilization in conjunction with the high density of glucocorticoid receptors in central fat compared to other areas of the body, the effect of cortisol on accumulation of fat is accentuated in this central area of the body resulting in a glucocorticoid-mediated redistribution of stored calories into abdominal fat (Bjorntorp, 2001). Bjorntorp argues that in statistical path analyses activity of the HPA axis is followed by waist-to-hip ratio and abdominal sagittal diameter indicating that both are dependent on elevated cortisol, and that these anthropometric measurements of centralization of body fat may serve as reasonable estimates of the long-term endocrine abnormalities associated with stress (Bjorntorp, 2001; Rosmond et al., 1998). The fat-accumulating effects of cortisol are not limited to central fat only, as similar analyses show that when BMI is included in such calculations leaving measurements of central fat (waist-to-hip ratio and sagittal abdominal diameter) out, BMI is closely following measurements of the HPA axis, suggesting that HPA axis activity is also involved in obesity in general (Rosmond et al., 1998).

In fact, the HPA axis and its end product cortisol are primarily concerned with energy intake, storage, and mobilization with long-term interrelationships among feeding, metabolism, energy storage, and glucocorticoid secretion (Dallman et al., 2004). Clinical observations in patients treated with corticosteroids as well as in patients with melancholic depression, a hypercortisolemic condition, indicate the co-occurrence of increased appetite, food intake, and obesity (Gold & Chrousos, 1999). There is interesting experimental evidence to demonstrate that increased cortisol responses to an acute laboratory stressor is predictive of voluntary increases in sweet, high-fat food ingestion after the stressor (Epel, Lapidus, McEwen, & Brownell, 2001). In this study, participants with high cortisol responses to the Trier Social Stress Test chose to eat more calories comprising sweet and fat foods than did low cortisol responders, whereas on the control day the two groups ate the same amounts. In a recent review (Dallman et al., 2004), Dallman maps a schematic of the regulation of feeding and the HPA axis. While acute stressors provoke transient increases in HPA axis activity with rapid feedback effects of glucocorticoids and immediate alteration of behaviors (such as high-sweet and fatty food intake (Epel et al., 2001)), chronic stress leads to an inhibition of negative feedback resulting in a glucocorticoid signal to promote further activation of the chronic stress response system with a prolonged presence of glucocorticoids and insulin acting to increase intra-abdominal caloric storage. Once intra-abdominal fat has increased through glucocorticoid-mediated redistribution of stored calories into abdominal fat, an unidentified signal from these fat stores acts on the brain to reduce the overall level of activity of the chronic stress response network, explaining the use of the term comfort food (Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004; Dallman et al., 2003).

Consistent with these suggested functions of intra-abdominal fat to be involved in reducing the activity of the chronic stress response network, intra-abdominal fat has been labeled an endocrine organ due to its ability to produce cortisol from its inactive precursor cortisone and to synthesize leptin which plays a central role in controlling body weight and regulating fat stores (Pantanetti et al., 2004). Hence, once the mechanism of glucocorticoid-mediated redistribution of stored calories into abdominal fat has developed, an increase in intra-abdominal fat stores as a consequence of elevated glucocorticoids together with insulin does not necessarily have to be due to increased glucocorticoids in the general circulation, because elevated glucocorticoids can be generated locally in intra-abdominal fat through conversion of cortisone to cortisol via the action of the enzyme 11-b-hydroxysteroid dehydrogenase type-1 which is produced abundantly in intra-abdominal fat (Masuzaki et al., 2001).

Variation in stimulated cortisol levels as well as in body fat distribution and adiposity is known to be partly heritable. A recent study using two different statistical methods (familial cross-trait correlations and bivariate segregation analysis) suggests common familial components underlying cortisol and body fat covariation with common polygenic but not major gene determinants accounting for 16% to 20% of the phenotypic variances in white families (Feitosa et al., 2002). Evidence for polygenic pleiotropy between cortisol levels and abdominal fat was only apparent without adjusting for fat mass. In sum, there are different pathways through which HPA axis function and central adiposity may be associated including glucocorticoid action on visceral fat, glucocorticoid action on appetite, and polygenic pleiotropy between cortisol levels and abdominal fat, among others.

Following previous findings of increased cortisol reactivity and increased levels of abdominal fat in women at familial risk for breast cancer compared to women at population risk for the disease in two separate analyses (chapter two and chapter five), the goal of the present analysis was to explore associations between central adiposity and stress hormones in premenopausal women at different levels of familial risk for breast cancer. We were further interested in mean differences between the familial risk groups in the composite dependent variable of HPA axis hyperactivity (work cortisol) and abdominal fat (waist circumference adjusted for BMI).

Methods

For the present exploratory analyses, the general design and procedures are identical to those described in chapters two through four. This analysis includes premenopausal women with complete cortisol and anthropometric measurements only resulting in a sample size of n=160 for this analysis.

Statistical analyses

As previously described, for outliers on the dependent variable of cortisol an algorithm of four standard deviations above the mean for each study group was established, so that outliers were sought separately within each group (NoFR/LoFR/HiFR grouping). The total listwise deletions due to extreme values were six. Square root transformations were computed to normalize cortisol distributions. Bivariate correlations

between anthropometric measures and the HPA reactivity measure of urinary cortisol during work were computed, as well as linear regression models indicating part correlations for each variable following a literature indicating that waist circumference is a predictor for premenopausal breast cancer after controlling for BMI (Harvie, Hooper, & Howell, 2003a). Following previous approaches, delta cortisol values (work-nadir (home or sleep)) were also analyzed. Correlations were computed for all women together, as well as separated out by familial risk group. Last, multivariate analysis of variance was performed on work cortisol, BMI, and waist circumference with familial risk status (NoFR, LoFR, and HiFR) as the independent variable. To investigate the impact of familial risk status on the individual dependent variables, a Roy-Bargmann stepdown analysis was performed to analyze the additional effect of familial risk status on work cortisol after adjusting for abdominal fat.

Results

Bivariate correlations among all women indicated no significant associations between anthropometric measures and HPA reactivity measures (Table 1). Part correlations to identify the amount of variance accounted for by each predictor uniquely on levels of work cortisol and delta cortisol (work-nadir (home or sleep)) indicated highest contributions (4.2% and 4.4% for work cortisol and delta cortisol, respectively) by belonging to the high familial risk group. Waist circumference compared to BMI shows opposite and higher effects on work cortisol and delta cortisol levels among all

women together, which is even more pronounced in the NoFR group (n=103) analyzed separately (part r=0.104, and part r=-0.147 for BMI and waist circumference predicting work cortisol, respectively, and part r=0.117, and part r=-0.159 for BMI and waist circumference predicting delta cortisol, respectively), and the LoFR group separately (part r=0.120, and part r-=0.149 for BMI and waist circumference predicting work cortisol, respectively, and part r=0.049, and part r=-0.125 for BMI and waist circumference predicting delta cortisol, respectively). Interestingly, the direction of association changes within the HiFR group, albeit with very small effect sizes (part r=-0.042, and part r=0.006 for BMI and waist circumference predicting work cortisol, respectively, and part r=-0.20, and part r=0.074 for BMI and waist circumference predicting delta cortisol, respectively).

Multivariate analysis of variance was performed on three dependent variables: work cortisol, BMI, and waist circumference. With the use of Wilks' criterion, the combined dependent variables of work cortisol, BMI, and waist circumference were significantly different between familial risk groups ($F[6,310]=4.013$, $p=0.001$. To see, what work cortisol adds to abdominal fat (waist circumference controlled for BMI), Roy-Bargmann stepdown analysis was performed using waist circumference and BMI as covariates, and work cortisol as the dependent variable. There was a significant main effect of familial risk group on work cortisol levels after adjusting for abdominal fat ($F[2,155]=4.062$, $p=0.02$) with planned contrasts indicating a significant difference between HiFR and NoFR ($p=0.009$), but not between LoFR and NoFR ($p=0.112$).

Table 1: Unadjusted and part correlations between anthropometric measures and work cortisol among n=160 women at different levels of familial risk for breast cancer

Dependent Variable	BMI	Waist Circumference	LoFR vs. other	HiFR vs. other
Zero-order correlations:				
Work Cortisol (sqrt)	r=-0.08, p=0.11	r=-0.10, p=0.10	r=0.10, p=0.11	r=0.17, p=0.02
Delta Cortisol (sqrt)	r=-0.07, p=0.20	r=-0.07, p=0.18	r=0.06, p=0.23	r=0.19, p=0.01
Unique variance*				
Work Cortisol (sqrt)	part r=0.064 0.4%	part r=-0.102 1.0%	part r=0.124 1.5%	part r=0.206 4.2%
Delta Cortisol (sqrt)	part r=0.044 0.2%	part r=-0.072 0.5%	part r=0.093 0.9%	part r=0.209 4.4%

* squared part correlation

Discussion

Contrary to previous findings on positive associations between abdominal fat and increased cortisol responses, the present data does not support a positive association but rather a negative association between cortisol responses and abdominal fat (non significant) in a sample of women at different levels of familial risk for breast cancer. Congruent with the lack of association between the variables, there was a main effect of familial risk status on the composite of work cortisol, BMI, and waist circumference with a continuing effect of familial risk status on work cortisol after adjusting for abdominal fat. Interestingly, more consistent with previous work, analysis among the HiFR alone

indicated a positive contribution of waist circumference (adjusted for BMI) to delta cortisol levels, albeit with very low effect sizes.

The failure to replicate previous findings on positive associations between abdominal fat and cortisol reactivity in the full sample could be due to differences in the samples studied, as well as differences in methodologies across studies. While most of the studies which report associations between abdominal fat and cortisol responses do not control for overall adiposity (Bjorntorp, 2001; Kopelman et al., 1988; Ljung et al., 1996; Marin et al., 1992; Pasquali et al., 1993; Rosmond, Dallman, & Bjorntorp, 1998; Rosmond et al., 2000), making it impossible to specifically disentangle associations of cortisol with obesity and/or central fat localization, only one study, to our knowledge, looked at abdominal fat and cortisol responses in lean women (Epel et al., 2000), but they found support for a positive relationship between higher waist-to-hip ratios and higher levels of cortisol during stress, as well as a lack of habituation to stress. In contrast to studies on abdominal fat and stress reactivity, we based our assessment method of abdominal fat on epidemiological studies on abdominal fat as a risk factor for breast cancer. In a systematic review, Harvie and colleagues (Harvie, Hooper, & Howell, 2003b) reported that abdominal fat as a risk factor for premenopausal breast cancer was best operationalized by waist circumference controlling for BMI. This finding is consistent with reports indicating an increase in visceral fat with increases in the waist circumference category within each of the three BMI categories studied (Janssen, Heymsfield, Allison, Kotler, & Ross, 2002). A further difference between our study and other studies that have investigated an association between central adiposity and cortisol levels, is the assessment of urinary cortisol during work as the stressful period of the day,

based on earlier studies indicating that it is the work place that consistently elicits the strongest stress responses of the day (i.e., Brown & James, 2000; James & Brown, 1997; Kario, James, Marion, Ahmed, & Pickering, 2002). Because urinary cortisol is a summary index of secretion of cortisol during the specific time period of urine collection, it is not sensitive to momentary changes occurring over the course of an hour. However, Epel and colleagues previously found increased cortisol levels during a stress session with larger areas under the curve for a time period of about three hours in lean women with high waist-to-hip ratios compared to lean women with low waist-to-hip ratios (Epel et al., 2000), an effect which could have been detected in a summary index of urinary cortisol during that time period.

By considering work as a stressful period of the day, we used a stressor that is most likely familiar, which could lead to different results than using a novel laboratory stressor, such as the Trier Stress Test. Epel and colleagues reported higher cortisol levels in response to both novel stress (Trier Stress Test on day one) and familiar stress (Trier Stress Test on two more days) in women with high waist-to-hip ratios compared to lean women with low waist-to-hip ratios (Epel et al., 2000), supporting our hypothesis (which we did not confirm) of finding such a relationship in our sample of working women. Interestingly, in Epel and colleagues study, threat appraisals of the challenge partially mediated the relationship between central adiposity and cortisol responses. Additionally, Epel and colleagues sample consisted of women in the lean/high waist-to-hip ratio category with significantly lower self-esteem, higher levels of pessimism, chronic work/financial stress levels, negative affect and problem avoidant coping, psychological

characteristics that may have mediated the relationship between abdominal fat and cortisol responses and that may be different in our sample.

The present analysis has limitations that must be recognized. First, we did not match the three familial risk groups on BMI resulting in a statistical trend toward mean overall adiposity differences between the risk groups with highest values in the NoFR group and lowest values in the HiFR group and a range of BMI values from 18 (underweight) to 51 (extremely obese). While we statistically control for BMI, previous results have indicated much weaker differences in cortisol by waist-to-hip ratio among overweight women (Epel et al., 2000) indicating the need for a less heterogeneous sample with regard to overall adiposity to detect associations between cortisol and abdominal fat. However, when we eliminated women who were obese (BMI values $\geq$30), the same pattern of results was apparent. Second, a proper test of a possible moderating effect of familial risk status on the relationship between abdominal fat and cortisol (i.e., through polygenic pleiotropy between cortisol levels and abdominal fat (Feitosa et al., 2002) would only have been possible with larger cell sizes in the low and high familial risk groups.

In sum, in contrast to previous findings, the results of the present data suggest negative associations of urinary cortisol during work with abdominal fat (when controlling for BMI), explaining 1% of the unique variance of waist circumference on work cortisol levels (not significant) compared to 4.2% explained by belonging to the high familial risk group. There is some indication for a moderating effect of familial risk status on this relationship with positive associations between waist circumference (controlled for BMI) and delta cortisol levels, albeit with small effect sizes.

Future studies should investigate the underlying mechanisms of the apparently independent concurrence of increased cortisol reactivity and increased levels of central adiposity (controlled for general adiposity) in women at high familial risk for breast cancer.

General Discussion

The goal of the present work was to investigate HPA axis function, psychosocial correlates, and central adiposity in women at familial risk for breast cancer with the aim of discovering a possible pathway through which stress and its hormonal correlates and possible associations with central adiposity may play a crucial role in the etiology of breast cancer for women with family histories of the disease. A series of experiments were presented: first, I showed elevated cortisol responses to daily stress (work) in working women at familial risk for breast cancer compared to women at population risk for the disease; second, I demonstrated that in the group of women at population risk for the disease breast cancer specific distress was related to cortisol responses to daily stress (work); third, I showed a gradient increase of cortisol levels in response to the work environment from no, low, to high familial risk (according to menopausal status at diagnosis of the first-degree relative) in a sample of premenopausal working women with conclusive evidence for intrusion effects on cortisol, but uncertainty about a mediation of group effects on cortisol by intrusions due to a significant intrusion effect in the full model rendering the familial risk group non-significant, but a lack of association between intrusions and cortisol in the low and high familial risk group separately, as well as a significant difference between low and high familial risk on intrusions, but not on work cortisol levels; fourth I presented results on increased levels of central adiposity (controlling for overall adiposity) in women at high familial risk of breast cancer compared to the low and no risk groups; and fifth, in an exploratory analysis of associations between cortisol responses and central fat, I found some indication for

negative associations between central fat and cortisol responses in the full sample, but positive associations in the high risk group alone.

Given the pattern of findings, the familial breast cancer model proposed in this work could be supported in large part by the empirical findings. While I showed support for increased stress reactivity and increased levels of central adiposity in women at high familial risk for breast cancer compared to women at population risk for the disease, the proposed relationship between stress reactivity and central adiposity, through which I originally established the etiological pathway to the development of breast cancer based on a systematic review and meta-analysis (Connolly et al., 2002; Harvie, Hooper, & Howell, 2003) concluding that central fat is a risk factor for breast cancer, did not find support in the present data (see chapter 7 for a discussion of the results). Though, due to the cross-sectional design of this study and the fact that the subjects in this study were healthy women not diagnosed with breast cancer but at different familial risks for the disease, the etiological significance of increased levels of central fat for the development of breast cancer can only be assumed by drawing on existing epidemiological studies, such as those reviewed by Harvie and colleagues, as well as Conolloy and colleagues.

In fact, studies investigating a possible link between stress and breast cancer risk have also existed for decades, albeit with less conclusive evidence (McKenna, Zevon, Corn, & Rounds, 1999; Duijts, Zeegers, & Borne, 2003) (for review). As Reiche and colleagues (Reiche, Nunes, & Morimoto, 2004) nicely review, the idea of a possible connection between psychological states and the outcome of human disease is old, dating back to AD 200, when Galen wrote that melancholic women were more susceptible to “swellings” of the breasts than were sanguine women (Dunn, 1996). The mechanisms of

how psychological stress can lead to a down-regulation of various parts of the cellular immune response through hormonal stress correlates, such as cortisol, has expanded greatly (Reiche, Nunes, & Morimoto, 2004). A causal model in which the relation between stress, depression, and carcinoma is clarified was proposed by Holden and colleagues (Holden, Pakula, & Mooney, 1998). In brief, stress is associated with increased expression of interleukin-1 beta and tumor necrosis factor-alpha, and reduced expression of interleukin-2, interferon-gamma, major histocompatability complex class II molecules and natural killer cell activity, all cellular immunity effects that are enhanced by glucocorticoids (Elenkov & Chrousos, 2002) (for review). Reiche and colleagues summarize that the majority of organ-related carcinomas are associated with elevated tumor necrosis factor-alpha. Tumor necrosis factor-alpha inhibits the activity of protein tyrosine phosphatase which leads to a diminished expression of the major histocompatability complex class I antigen on the cell surface allowing malignant cells to escape immune surveillance. In sum, according to this model, stress can foster tumor progression by means of inhibiting the expression of major histocompatability complex class I and II molecules and through the reduction of natural killer cell activity. Reiche and colleagues further state that there may be a path from stress to cancer through events that modulate the development and accumulation of somatic mutations and genomic instability, such as increases in DNA damage, alterations in DNA repair, and inhibition of apoptosis (Reiche et al., 2004). Recent intriguing evidence for life stress effects on chromosomal stability and biomarkers of cell aging stems from results indicating that women with the highest levels of perceived stress have telomeres (DNA-protein complexes that cap chromosomal ends, promoting chromosomal stability) shorter on

average by the equivalent of at least one decade of additional aging compared to low stress women (Epel et al., 2004).

However, while, on a theoretical level, pathways for stress to breast cancer have been well formulated, empirical data is contradictory and has been characterized by weak designs (Reiche et al., 2004). Several narrative reviews (Bryla, 1996; Butow et al., 2000; Cox & Mackay, 1982; Gerits, 2000; Geyer, 2000) as well as meta-analyses have attempted to summarize and quantify the effects of psychosocial factors on the development of breast cancer (Duijts et al., 2003; McKenna et al., 1999; Petticrew, Frase, & Regan, 1999). Due to the methodological diversity of the observational studies that entered the reviews, consisting of differences in design, adjustment for confounding, population characteristics, and effect measures, the reviews that have been published on the relationship between stressful life events or emotional factors and breast cancer risk tend to fall into two categories: 1) those that concluded there is no association because of methodologic differences (Gerits, 2000; Petticrew et al., 1999), and 2) those that supported a modest association (Bryla, 1996; Butow et al., 2000; Cox et al., 1982; Geyer, 2000; McKenna et al., 1999; Wenderlein, 1978).

McKenna and colleagues meta-analysis identified four of eight psychosocial factors that were significantly more prevalent in breast cancer patients compared to controls, namely denial/repression coping in response to life stressors, experience of separation or loss, a history of stressful life experiences and conflict avoidant personality, but not anxiety/depression, childhood family environment, anger expression, and extraversion-introversion (McKenna et al., 1999). There are several problems with retrospective case-control studies and the conclusion that psychosocial factors play a part

in the development of the disease. The retrospective design does not allow for the precedence of these factors over the diagnosis of the disease, specifically for state-like constructs such as coping styles, which may be influenced by the experience of the disease. Also, the retrospective recall of life events may be biased in breast cancer patients compared to control subjects due to a motivation of finding the origin to the illness and making sense of the diagnosis. For example, Lavery and colleagues demonstrated that 70% of breast cancer patients made causal attributions about their cancer's origins (controllable and uncontrollable) (Lavery & Clarke, 1996). Hence, prospective designs are necessary to draw causal conclusions of psychosocial factors playing a part in the development of breast cancer.

A more recent meta-analysis on the relationship between stressful life events and breast cancer risk aimed to quantify the association for various categories of stressful life events (e.g., stressful life events, death of spouse, death of relative or friend, personal health difficulties, non-personal health difficulties, change in marital status, change in financial status and change in environmental status) (Duijts et al., 2003). After investigating qualitative and quantitative data from 27 studies, out of which 17 were prospective designs, the authors concluded that only the categories stressful life events, death of spouse, and death of relative or friend showed a statistically significant effect, albeit with death of spouse being the only category without publication bias and without sources of heterogeneity. Interestingly, for the categories of death of spouse and death of relative or friend, the retrospective studies showed less strong association parameters (odds ratios) than the prospective studies. For the category of stressful life events, only 3 studies were prospective with odds ratios ranging from barely over 1 (Lillberg et al.,

2003) to almost 3 (Chen et al., 1995). In sum, the widespread belief and theoretically well formulated connection between personality or stress and the development of breast cancer can only be partially supported by studies investigating a link between psychosocial factors and the development of breast cancer. More well-designed prospective studies are needed to provide conclusive evidence of the contribution of stress and personality to the development of breast cancer.

To our knowledge, none of the studies reviewed above on psychosocial factors and the development of breast cancer assessed biological mediators such as hormonal and immune stress correlates and/or cellular events. For a more in depth understanding of how psychosocial factors relate to disease development, McEwen specified a heuristic model on the relationship between chronic stress and disease suggesting the elements of a) allostatic load defined as the wear and tear that the body experiences due to repeated cycles of having to maintain stability through change (allostasis) in response to stressful experiences, b) primary mediators defined as chemical messengers that are released as part of allostasis (e.g., cortisol, noradrenaline, epinephrine, and DHEA), c) primary effects defined as cellular events (structural proteins, enzymes, receptors and ion channels) all regulated as part of allostasis by the primary mediators, d) secondary outcomes defined as the cumulative outcome of the primary effects in a tissue/organ specific manner in response to the primary mediators (e.g., abdominal fat, blood pressure, cholesterol/HDL ratio), and e) tertiary outcomes defined as the actual diseases or disorders which are the result of the allostatic load (McEwen & Seeman, 1999)). In brief, the allostatic load model postulates four conditions that lead to allostatic load: 1) repeated hits from multiple novel stressors, 2) lack of adaptation to a stressor, 3) prolonged

response to a stressor due to delayed shut down, and 4) inadequate response to a stressor leading to compensatory hyperactivity of other mediators.

The results of our studies demonstrating increased psychological distress (perceived stress), increased cortisol levels in response to work stress (primary mediators), and increased levels of abdominal fat (secondary outcomes) in women at familial risk for breast cancer compared to population risk women are entirely consistent with an indication of the presence of allostatic load in these women. Our finding of increased cortisol levels in response to work stress is one of the two major subtypes of the HPA axis response to stress besides the other type of mild hypocortisolism, as recently suggested by Hellhammer and colleagues (Hellhammer et al., 2004). Interestingly, when they compared individuals with and without hypocortisolism on a cumulative allostatic load index consisting of blood pressure, waist-to-hip ratio, serum high density lipoproteins (HDL), cholesterol/HDL ratio, serum dehydrooepiandrosterone sulfate (DHEA-S), fibrinogen, C-reactive protein, and blood plasma levels of glycosylated hemoglobin (HbA1c), they found lower allostatic load scores, but higher depression, perceived stress, and physical complaint scores in hypocortisolemic individuals. These results suggest a protective role of the hypocortisolemic stress response on allostatic load indices mainly presenting risk factors for metabolic and cardiovascular disease.

With regard to the pattern of allostatic load, the results of increased urinary cortisol levels in response to work stress could be classified as a lack of adaptation, a prolonged response, or repeated hits. Due to the time-integrated assessment of the stress response system by urinary cortisol collection, the pattern exhibited by the women at familial risk for breast cancer in this study cannot be identified. Selective time points

across a period of time using plasma or saliva sampling would allow for the identification of the particular type of allostatic load in women at familial risk for breast cancer.

Allostatic load indices have been related to health problems in various domains, including cardiovascular function and psychiatric illness (McEwen et al., 1999; McEwen, 2003; McEwen, 2004). A hyperactive HPA axis accompanies depressive symptoms as demonstrated by elevated circulating plasma levels of corticotropin (ACTH) and cortisol, as well as elevated urinary cortisol levels (Rubin et al., 1987; Rubin, Poland, Lesser, Winston, & Blodgett, 1987), and elevated levels of corticotropin releasing hormone (CRH) in the cerebrospinal fluid (Nemeroff et al., 1984). Seligman described the HPA activated state as learned helplessness or as a defeat and depressive condition (Holsboer & Barden, 1996) (for review). Not only is a hyperactive HPA axis associated with depressive symptoms, but also does it appear to play a crucial role in the occurrence and remission of the symptoms, as a normalization of HPA system dysregulation seems to be a necessary co-factor for clinical remission of depressive symptoms and remaining hypersecretion of cortisol after treatment can be a predictive factor for an increased risk for relapse or recurrence of depression (Zobel et al., 2001; Zobel, Yassouridis, Frieboes, & Holsboer, 1999). Hence, this work's finding of increased cortisol levels in response to work stress in women at familial risk for breast cancer, which is consistent with a previous report indicating increased cortisol responses to a laboratory stressor in women at familial risk for breast cancer (Gold, Zakowski, Valdimarsdottir, & Bovbjerg, 2003), may indicate a greater health risk not only for breast cancer, but also for mood disorders in women at familial risk for breast cancer.

Further indication for a greater health risk other than the development of breast cancer lays in this work's finding of increased central fat adjusted for overall adiposity in women at familial risk for breast cancer. Central fat, independent of body mass index, has been related to type 2 diabetes mellitus and cardiovascular disease in cross-sectional and longitudinal studies (Pi-Sunyer, 2004) (for review). In sum, it appears that there is good reasoning for the possibility that having a family history of breast cancer poses a health risk beyond that of developing breast cancer. To my knowledge, studies on health risks other than breast cancer in women with family histories of breast cancer do not exist. Large epidemiological studies are needed to investigate the risk for serious health problems such as the metabolic syndrome (a diagnostic category to identify individuals at increased risk of cardiovascular disease consisting of abdominal obesity, impaired fasting glucose, high blood pressure, high levels of triglycerides paired with low levels of HDL-C indicating insulin resistance (Reaven, 2005)), cardiovascular disease, or psychiatric illnesses in the presence of a family history of breast cancer. Furthermore, future studies should capture further allostatic load elements to investigate the prevalence of allostatic load measures specifically related to the development of breast cancer compared to measures more indicative of the development of other serious health issues, as mentioned above, in women with family histories of breast cancer.

The allostatic load model provides a possible explanation of the mechanisms underlying the increased work cortisol responses and increased central fat levels in women at familial risk for breast cancer, though alternative mechanisms to explain the phenomena exist. The results of our analyses indicated intrusion effects on work cortisol levels albeit with inconclusive evidence for a mediation of group effects on cortisol by

intrusions. Further mechanisms not related to perceived stress must exist. Recent research has studied the impact of genetic factors on different aspects of HPA functioning. While evidence from quantitative genetic studies document a significant impact of genetic factors on basal HPA axis function with a heritability of 62% in an analysis of five comparable twin studies (Bartels, Van den, Sluyter, Boomsma, & de Geus, 2003), investigations of the heritability of stimulated HPA axis activity is scarce and inconsistent. Three out of four twin studies argue against a substantial contribution of genetic factors on variation in stimulated cortisol and ACTH levels (Froehlich, Zink, Li, & Christian, 2000; Nurnberger, Jr. et al., 1982; Inglis et al., 1999), and only one twin study suggests a moderate genetic effect on salivary cortisol responses to a psychosocial stressor (Kirschbaum, Wust, Faig, & Hellhammer, 1992). A recent twin study attempted to take into consideration different environmental settings by repeatedly exposing participants of the study to the same psychosocial stressor inducing a contextual change from a high anxiety/high novelty situation to a low anxiety/low novelty situation and found increasing heritabilities of salivary cortisol, total cortisol, ACTH, and heart rate responses after repeated exposure to the same psychosocial stressor (Federenko, Nagamine, Hellhammer, Wadhwa, & Wust, 2004). This finding suggests the possibility for a heritability of higher cortisol responses to daily stress in women at familial risk for breast cancer, assuming that the work environment is a stressor that the women are facing repeatedly and which is not likely to be novel. However, the habituation pattern of cortisol responses to repeated stressors has been described previously (Kirschbaum et al., 1995; Schommer, Hellhammer, & Kirschbaum, 2003), and may be viewed as a decrease in the effectiveness of the laboratory stressor rather than the manipulation of the

environmental context of the psychosocial stressor. Even though the laboratory stressor exposure resulted in significant HPA axis responses on all three test days, the fact that increased state-anxiety levels were only observed in response to the first but not to the second and third laboratory stress test exposure, which was explained as the contextual change from high to low anxiety by the authors, may indicate an absence of a stressful experience for the participant. The significant time effect could then be due to speech and mental activity rather than the experience of stress with aspects of uncontrollability and social evaluation (Dickerson & Kemeny, 2004). This idea is further supported by the fact that baseline levels on the first test day were almost as high as peak levels on the second and third test day. Higher heritability levels at later time points would then be consistent with the more congruent reports on the impact of genetic factors on basal HPA axis (Bartels et al., 2003). Interestingly, a separate analysis with a highly overlapping sample (four twin pairs less) indicated no evidence for an impact of genetic factors on the individual habituation pattern of cortisol responses to repeated stress exposure (Wust, Federenko, Van Rossum, Koper, & Hellhammer, 2005).

In contrast to the majority of the quantitative genetic studies, association studies indicate associations between common polymorphisms in the glucocorticoid receptor genes and adrenocortical response to psychosocial stress (Wust et al., 2004). Several glucocorticoid receptor gene polymorhpisms have been associated with endocrine or metabolic measures with particularly interesting findings for the BClI restriction fragment length polymorphism in the GR gene which has been found to be associated with cortisol responses to a standardized lunch (Rosmond et al., 2000), as well as with visceral fat, waist to hip ratio, and body mass index (BMI) (Buemann et al., 1997;

Rosmond et al., 2000). The same is valid for the 363S allele which has been associated with a higher BMI (Lin, Wang, & Morris, 1999), a higher waist to hip ratio (Dobson, Redfern, Unwin, & Weaver, 2001), and increased salivary cortisol responses to acute psychosocial stress (Wust et al., 2004). Feitosa and colleagues (Feitosa et al., 2002) report polygenic pleiotropy (several genes have an influence on several characters between cortisol levels and abdominal fat in white families. These findings are particularly relevant to our findings of increased cortisol responses to the work environment, and higher levels of abdominal fat in women at familial risk of breast cancer compared to women without a familial risk of the disease. Future studies should consider these two identified gene polymorphisms as underlying pathophysiological role for the heightened cortisol responses and increased levels of abdominal fat in women at familial risk for breast cancer.

Consistent with the idea of an underlying polygenic pleiotropy for the effects of a family history of breast cancer on cortisol responses to work and central fat levels with an unspecified link to the genetics of breast cancer development, the data supported a ranking of familial risk according to the age at diagnosis of the first-degree relative with highest levels of psychological distress, stress hormone levels, and central adiposity levels in women at high familial risk for the disease. In a hypothetical polygenic model in which many genes with only a weak contribution individually may additively or multiplicatively contribute to increased risk of breast cancer among women with family histories of the disease (through effects on several breast cancer risk factors), it is tempting to propose that the high levels of psychological distress (intrusive thoughts about the disease) are induced and sustained by high glucocorticoid levels an the

induction of elevated corticotropin releasing hormone production in the central nucleus of the amygdala, a site of the brain which when stimulated increases the likelihood that events would be perceived as fearful and the individual perhaps ridden with anticipatory angst (Schulkin, McEwen, & Gold, 1994; Schulkin, Gold, & McEwen, 1998).

In sum, both the allostatic load model and the hypothetical polygenic model find applications to the present data and each has the theoretical capability of providing a greater framework of underlying mechanisms and links to the findings of greater psychological distress, increased cortisol responses to work, and increased levels of central fat in women at familial risk for breast cancer compared to women at population risk for the disease. Future studies are needed to provide empirical evidence for both models in their application to familial breast cancer risk.

The present study has limitations that must be recognized. We used urinary free cortisol collection to investigate stress hormone levels in a naturalistic design where the workday was divided into three different microenvironments: work, home and sleep, where work was operationally defined as the stressor condition, based on earlier studies indicating that it is the work place that consistently elicits the strongest stress responses of the day (e.g.,(Brown & James, 2000; James & Brown, 1997; Kario, James, Marion, Ahmed, & Pickering, 2002). Urinary cortisol collection was chosen over plasma or salivary cortisol in this study because a) it permits a time-integrated assessment of the stress response system rather than selective time points across a period of time as is captured only with plasma and salivary cortisol, and b) it is a noninvasive procedure for the collection of hormone levels and, hence, does not have an impact on the concentrations like a potentially as stressful perceived blood drawing can have on plasma

cortisol. However, problems with urinary collection include a) compliance can be difficult, b) as with plasma and salivary cortisol, time-integrated hormone production is influenced by sleep-wake cycle, physical activity, and meals, among other, and 3) values may be influenced by hydration status (Masi, Rickett, Hawkley, & Cacioppo, 2004) (for review). Consecutively, diurnal fluctuation of cortisol levels and a possible influence of hydration status on hormone levels will be discussed in more detail.

Cortisol excretion rates underlie a distinct circadian rhythm with a morning maximum, declining levels throughout the daytime, a period of low concentrations generally centered around midnight, and a rise after the first few hours of sleep (Weitzman et al., 1971). Given this pattern of cortisol excretion over the course of a day, timing of sample collection is of high importance. The goal of the present study was to investigate urinary cortisol excretion rates during stress applying a naturalistic study design by dividing the day into three time blocks: work, home, and sleep. Therefore, collection times inevitably varied across participants raising the question of whether our results of higher work cortisol levels among women at high familial risk could be confounded by earlier collection times in this group of women. While analyses indicated a seven hour range of starting time (8AM to 3PM), the vast majority of subjects (86.4%) started their collection time between 10AM and 12PM, and ended it between 2PM and 5PM (90.9%); group differences in start time, end time, and collection duration were not apparent (Appendix D). We conclude that our results are unlikely to be confounded by circadian effects of cortisol excretion.

There has been some debate over whether urinary free cortisol excretion is influenced by fluid intake (Fenske, 2004) (for review). In our sample, there was no

difference between urine volume during work between women at high familial risk for breast cancer compared to women at no familial risk for the disease; women at low familial risk showed increased urine volumes during work compared to no familial risk women. Our results indicated no significant difference between low familial risk women and no familial risk women on urinary free cortisol levels, however, descriptives statistics suggested that a larger sample size may identify higher cortisol levels in low familial risk women compared to no familial risk women. However, it is unlikely, that our results are biased by the higher urinary volume among low familial risk women. Considerable work in humans has studied the influence of increased fluid ingestion on the excretion of free glucocorticoisteroids in urine. While earlier studies found a stimulatory influence of water diuresis on urinary free cortisol in healthy individuals (HATFIELD & SHUSTER, 1959; Baum, Davison, & Landon, 1974; Bertrand, Rudd, Weller, & Day, 1987; Mericq & Cutler, Jr., 1998), more recent evidence suggests that urinary free cortisol excretion is not increased during water diuresis (Lewicka, Nowicki, & Vecsei, 1998; Fenske, 2004; Putignano, Dubini, & Cavagnini, 2000). In comparison to older studies, more recent work has used more specific methods such as chromatography/RIA and HPLC to measure urinary free cortisol. Consistent with the idea of measurement discrepancies between nonspecific and more specific protein binding assays, previous investigations consistently measured higher urinary free cortisol amounts compared to more recent studies. Fenske (Fenske, 2004) suggests that previous studies claiming a positive relationship between urinary free cortisol and urine volume should be regarded with caution because urinary free values in these studies may represent the sum of cortisol and cortisone due to nonspecific protein assays measuring the urinary excretion of a cortisol

precursor/metabolite rather than cortisol itself. In sum, it is unlikely that our results are confounded by sleep-wake cycle or hydration status.

A further limitation of this study is related to the stressor condition in our naturalistic design. The design of this naturalistic experimental study approach considering work as the stressful period of the day is based on previous reports indicating that it is the work place that consistently elicits the strongest stress responses of the day (e.g.,(Brown et al., 2000; James et al., 1997; Kario et al., 2002). While reports do not support a reliable association between self-reported stress or negative states and stress hormone levels, with some studies indicating an increase of cortisol in response to negative states (Lundberg & Frankenhaeuser, 1980), and others not (Hubert & Jong-Meyer, 1991b; Hubert & Jong-Meyer, 1991a), a limitation of our study, nevertheless, is the lack of assessment of perceived stress during work and home to confirm the stressor condition (work) against the non-stressful condition (home) of our naturalistic experimental design. However, preliminary data analysis of mood ratings collected using a diary approach with assessments every 15 minutes in a subsample of 116 women indicated a higher percentage of recordings for anxious during work compared to home and no difference between the study groups on this variable. The fact that there was no association between the amount of anxiety during work and work cortisol levels in our data is consistent with results from Hubert and deJong-Meyer (Hubert et al., 1991b; Hubert et al., 1991a), who did not find cortisol changes with anxiety inducing film stimuli. This disparity in self-reported distress and concurrent cortisol levels is a recognized fact in the literature (Lutgendorf, Reimer, Schlechte, & Rubenstein, 2001; Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005).

Overall, participants of our studies were quite diverse with 43.2% being non-white. While we consider this a strength of our sample, it is important to consider ethnic differences in HPA axis function. Very few studies have investigated possible ethnic differences in stress hormone excretion levels. One such study reported significantly lower awakening cortisol in saliva among African-American participants with the effects being independent of perceived stress (Bennett, Merritt, & Wolin, 2004). Another study reported no racial differences in 24-h urinary free cortisol excretion, dexamethasone suppressibility of plasma cortisol, baseline plasma cortisol and ACTH concentrations, or plasma cortisol response to CRH (Yanovski, Yanovski, Gold, & Chrousos, 1993). A later study by the same research group also reported no differences in plasma cortisol before and after exercise (as physiological stimulus for ACTH secretion) between African American and Caucasian women (Yanovski et al., 2000). A recent study by Masi et al. which specifically addressed ethnic differences in urinary stress hormones in a population-based study confirms previous results of a lack of differences in cortisol production by ethnicity (Masi et al., 2004). The authors point out that a number of studies have reported ethnic differences in creatinine levels, which is the most common method of correcting for effects of hydration status when measuring urinary hormone concentrations. Hence, creatinine-correction leads to underestimation of urinary hormone values among blacks compared to whites. Despite the fact that our sample is quite diverse, it is unlikely that our results are biased by ethnic differences in hormone levels. We employed a volume adjustment rather than a creatinine adjustment to avoid the problems accompanied by creatinine standardization. We further investigated ethnic

differences between the familial risk group and correlations between ethnicity/race and cortisol levels to evaluate for possible confounding.

Future studies are needed to investigate the increased health risk in women at familial risk for breast cancer, as well as the underlying mechanisms to the risk. In addition to the already mentioned research needs of a) assessing other health risks in women at familial risk for breast cancer, b) including more allostatic load measures (primary mediators, primary effects, and secondary outcomes), and c) including gene polymorphisms in future studies, it is important to apply a longitudinal design to investigate who develops breast cancer and other diseases and at what age the adverse health effects occur. Also, the possibility of a higher prevalence of other well established risk factors for breast cancer in women at familial risk for breast cancer, such as early age at menarche, nulliparity, late onset of menopause, proliferative benign breast pathology, and mammographic density should be investigated in future studies, as well as possible underlying mechanisms for an increased prevalence of these risk factors. Last, for the continuation of the study of increased stress reactivity in women at familial risk for breast cancer, further possible mediators and moderators, such as caregiving for the diagnosed relative, death of the first-degree relative, and other psychological variables including cognitive phenomena (i.e., illness attributions), personality characteristics (i.e., optimism), and health behaviors (i.e., alcohol consumption and exercise), should be considered.

In conclusion, our results of a series of analyses indicate an increased health risk for women at familial risk of breast cancer, specifically those at high familial risk (first-degree relative diagnosed at premenopausal age). The data presented in this work

indicates two different pathways through which the increased health risk in women at familial risk for breast cancer occurs: elevated cortisol responses to daily stress (work), and elevated levels of abdominal but not general obesity. Both of these conditions have been related to detrimental health effects with cortisol mediating effects ranging from induction of liver enzymes involved in energy metabolism to regulating the trafficking of immune cells and cytokine production (Chrousos, 2000; McEwen et al., 1999; Vanitallie, 2002), and abdominal fat adjusted for general obesity being related to breast cancer (Harvie et al., 2003), as well as type 2 diabetes mellitus and cardiovascular disease in cross-sectional and longitudinal studies (Pi-Sunyer, 2004) (for review). Future studies are needed to investigate the underlying mechanisms of both conditions to determine whether prevention strategies such as stress management and exercise training may be beneficial for these women.

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PERSONAL DATA

1. Today's date: ___ ___/ ___ ___/ ___ ___ (m/d/y)

2. Birth date: ___ ___/ ___ ___/ ___ ___ (m/d/y)

3. Height: _____ (ft) _____ (in)

4. Weight: _____ (pounds)

5. Ethnic group (circle one number):

   1 White (non-Hispanic)
   2 White (Hispanic)
   3 Black (non-Hispanic)
   4 Black (Hispanic)
   5 Asian/Indian
   6 Asian or Pacific Islander
   7 Native American
   8 Other _____
   9 Unknown

6. Marital status (circle one number):

   1 Never married
   2 Currently married
   3 Separated
   4 Divorced
   5 Widowed

7. Who lives with you? (circle all that apply):

   1 No one
   2 Spouse or partner
   3 Roommate(s) (not a partner)
   4 Parent(s)
   5 Children
   6 Other relatives
   7 Other _____

8. How long have you lived with the people you live with now? (circle one number):

   1 Less than 1 month
   2 One to 6 months
   3 Seven months to 2 years
   4 Two to 5 years
   5 More than 5 years

9. Level of school completed? (circle one number):

   1 Less than 7th grade
   2 Junior High school (9th grade)
   3 Partial high school (10th or 11th grade)
   4 High school graduate
   5 Partial college or specialized training
   6 Standard college or university graduate
   7 Graduate professional training (graduate degree)

10. Current employment situation (circle one number):

A. WORKING

1 Full time at job
2 Part time at job

B. ON LEAVE

3 On leave with pay
4 On leave without pay

C. NOT EMPLOYED

5 Seeking work
6 Not seeking work
7 Receiving disability
8 Not self-supporting
9 Homemaker
10 Retired

D. STUDENT

11 Full time
12 Part time

11. Which category best describes your occupation? If you are not currently employed, which best describes your **LAST** job? If you are a homemaker, which best describes your spouse's usual occupation? (circle one number)

1. Professional, Technical, & Related Occupations (as teachers/professors, nurses, lawyers, physicians, & engineers)
2. Manager, Administrator, or Proprietor (as sales managers, real estate agents, or postmasters)
3. Clerical & Related Occupations (as secretaries, clerks or mail carriers)
4. Sales Occupations (as sales persons, demonstrators, agents & brokers)
5. Service Occupations (as police, cooks, or hairdressers)
6. Skilled Crafts, Repairer, & Related Occupations (as carpenters, repairers, or telephone line workers)
7. Equipment or Vehicle Operator & Related Occupations (as drivers, railroad brakemen or sewer workers)
8. Laborer (as helpers, longshoreman, or warehouse workers)
9. Farmer (owners, managers, operators or tenants)
10. Member of the military
11. Other (please describe) _____

12. Approximate annual gross income for your household: (circle one number)

1 Less than $ 10,000
2 $10,000 - $19,999
3 $20,000 - $ 39,999
4 $40,000 - $59,999
5 $60,000 - $100,000
6 Greater than $100,000

(Remember, all information will be used for statistical purposes only)

Circle either "YES" or "NO"

13. Over the past several years how much sleep do you normally get each night?

_____ hr _____ min

14. Over the past several years how much sleep per night have you needed to feel at your best?

_____ hr _____ min

15. What religion do you consider yourself a member of (please write in answer, write “none” if appropriate)?

_____

16. During the past month, how many hours of *actual sleep* did you get at night? (This may be different than the number of hours you spend in bed).
Hours slept per night _____

17. During the past month, when have you usually gone to bed at night?
Usual bed time _____

18. During the past month, when have you usually gotten up in the morning?
Usual getting up time _____

19. During the past month, how many times do you usually wake up at night? (This includes getting up to use the bathroom.)
Number of times I wake up at night _____

20. During the past month, do you usually feel refreshed after you wake up this morning?

(Please circle one) **YES** **NO**

21. During the past month, how would you rate your sleep quality overall? (Please place an X next to the words which best describe your sleep.)

Very Good _____
Fairly Good _____
Fairly Bad _____
Very Bad _____

MEDICAL HISTORY

1. How many times have you been seen by a doctor during the past year for any reason? (check best answer)
   1 ☐ None 2 ☐ 1 time 3 ☐ 2-5 times 4 ☐ 6-12 times 5 ☐ over 12 times

2. When was the last time you had a complete physical examination?
   1 ☐ Within the last year 2 ☐ 1-2 years ago 3 ☐ 2-5 years ago 4 ☐ over 5 years ago

3. When was the last time you had a mammogram?
   1 ☐ Within the last year 2 ☐ 1-2 years ago 3 ☐ 2-5 years ago 4 ☐ over 5 years ago
   5 ☐ Never had one

4. During your lifetime, have you smoked at least 100 cigarettes (5 packs)?
   1 ☐ Yes 2 ☐ No **(Skip to Question 5)**

   If you answered YES to Question 4,
   a) At what age did you begin smoking regularly? _____ Age in years
   b) How many cigarettes do/did you regularly smoke each day? _____ Cigarettes

   c) Have you smoked in the past month?
   ☐ Yes, approximately _____ cigarettes per day.
   ☐ No, I quit approximately _____ years ago.

5. Have you consumed any alcoholic beverages in the past month?
   1 ☐ Yes 2 ☐ No **(Skip to Question 6)**

   If you answered YES to Question 5, which of the following best describes how many alcoholic beverages you consumed in the past month?

   (Note: Beer: 1 can = 1 drink; Wine: 1 glass = 1 drink; Hard Liquor: 1 shot = 1 drink)

   1 ☐ 1 drink a month
   2 ☐ 2-3 drinks a month
   3 ☐ 1-2 drinks a week
   4 ☐ 3-4 drinks a week
   5 ☐ 1 drink nearly every day
   6 ☐ 1 drink a day
   7 ☐ 2 drinks a day
   8 ☐ 3 or more drinks a day

6. To your knowledge, have you ever been exposed to asbestos, solvents, or other industrial chemicals? 1 ☐ Yes 2 ☐ No

7. Have you ever been disabled for more than 2 months? 1 ☐ Yes 2 ☐ No

8. Have you had surgery before? 1 ☐ Yes 2 ☐ No
   If yes, when? Date(s):_____
   For what? _____

9. Have you had a biopsy for any cancer? 1 ☐ Yes 2 ☐ No
   If yes, when? Date(s):_____
   For what? _____

ID # _____ Date _____

10. Have you ever had a disease lasting longer than 2 months? 1 ☐ Yes 2 ☐ No

If yes, when? Date(s):_____

What? _____

11. Do you take any medication or natural supplement regularly? 1 ☐ Yes 2 ☐ No

	Drug	Dose	How Often?	Since
(EXAMPLE)	Tylenol-Extra Strength	2 capsules	twice daily	June, 1995
(Pain)	_____	_____	_____	_____
(Heart)	_____	_____	_____	_____
(Birth Control)	_____	_____	_____	_____
(Hormones)	_____	_____	_____	_____
(Other)	_____	_____	_____	_____
(Other)	_____	_____	_____	_____
(Other)	_____	_____	_____	_____
(Other)	_____	_____	_____	_____

13. Are you now having or have you ever had:

Chemotherapy	1 ☐ Yes	2 ☐ No
Radiation therapy	1 ☐ Yes	2 ☐ No
Cortisone	1 ☐ Yes	2 ☐ No

14. Do you consider yourself (circle):

1 ☐ Premenopausal 2 ☐ Postmenopausal 3 ☐ Not sure

(Continue to get periods) (Do not get periods)

15. If premenopausal, what was the date of the first day of the last time you had menstrual bleeding? _____

16. In days, what is the typical length of your menstrual cycle? _____ days

ID # _____ Date _____

17. Below are some situations which can cause some people to feel nauseated and/or to vomit. Please indicate if any of these situations have made you feel nauseated or caused you to vomit by checking one or both columns.

	Nausea has occurred with this item	Vomiting has occurred with this item
Pregnancy	_____	_____
Motion sickness	_____	_____
Drinking alcohol	_____	_____
Anxiety	_____	_____
Odors (perfume, shaving lotion, etc.)	_____	_____
Cigarette smoke	_____	_____
Taking pain medicine	_____	_____
Watching someone else vomit	_____	_____
Sight of blood	_____	_____
Food items (e.g., eggs)	_____	_____
Surgery	_____	_____
Other	_____	_____

FAMILY HISTORY OF CANCER

We are interested in knowing as much as possible about cancer in your biological relatives. On the following form, please indicate your relatives, what type of cancers they had, how old they were at the time of their diagnosis, as well as your age at that time. Please answer to the best of your knowledge. Approximate ages are useful if you cannot be exact, for example, "60's or 70's". Put "?" if you are not sure.

NOTE: Please list separately each cancer for each biological relative. (Please see examples in shaded areas).

	First Cancer			**Second Cancer**				
Relative Code (see bottom)	Location or Type of Cancer	Their Age at Diagnosis	Your Age Then	Location or Type of Cancer	Their Age at Diagnosis	Your Age Then	Outcome: Died from cancer? Yes (Y) No (N)	Were Both Breasts Affected?
1	Breast	55	26	Ovarian	65	36	Y	No
6	Colon	40	18				N	

1 = your mother
2 = your sister
3 = your daughter
4 = mother's mother
5 = mother's father
6 = mother's sister
7 = mother's brother
8 = mother's first cousin
9 = other (on mother's side)
10 = your father
11 = your brother
12 = your son
13 = fathers' mother
14 = father's father
15 = father's sister
16 = father's brother
17 = father's first cousin
18 = other (on father's side)

FAMILY HISTORY OF CANCER

1. Were you living with any of the above family member(s) when they had cancer? (circle one): YES NO

   If yes, which family member(s)? _____;_____;_____
   If yes, how old were you then? _____;_____;_____

2. Did you take care of any of the above family member(s) (emotionally or physically) when they had cancer? (circle one): YES NO

   If yes, which family member(s)? _____;_____;_____
   If yes, how old were you then? _____;_____;_____

3. Have you had any friends with cancer? (circle one): YES NO

   If yes, what type of cancer? _____;_____;_____
   If yes, how many friends? _____

4. Were you living with any friends when they had cancer? (circle one): YES NO

   If yes, what type of cancer? _____;_____;_____
   If yes, how many friends? _____

5. Did you take care of any friends (emotionally or physically) when they had cancer? (circle one): YES NO

   If yes, what type of cancer? _____;_____;_____
   If yes, how many friends? _____

FAMILY HISTORY OF HEART DISEASE

We are interested in knowing as much as possible about heart disease in your biological relatives. On the following form, please indicate your relatives, what type of heart disease they had, how old they were at the time of their diagnosis, as well as your age at that time. Please answer to the best of your knowledge. Approximate ages are useful if you cannot be exact, for example, "60's or 70's". Put "?" if you are not sure.

NOTE: Please list separately each heart disease for each biological relative. (Please see examples in shaded areas).

	First Heart Disease			**Second Heart Disease**			
Relative Code (see bottom)	Location or Type of Heart Disease	Their Age at Diagnosis	Your Age Then	Location or Type of Heart Disease	Their Age at Diagnosis	Your Age Then	Outcome: Died from heart disease? Yes (Y) No (N)
11	Angina	50	40				Y
2	Hypertension	40	28	Heart attack	53	41	N

1 = your mother
2 = your sister
3 = your daughter
4 = mother's mother
5 = mother's father
6 = mother's sister
7 = mother's brother
8 = mother's first cousin
9 = other (on mother's side)
10 = your father
11 = your brother
12 = your son
13 = fathers' mother
14 = father's father
15 = father's sister
16 = father's brother
17 = father's first cousin
18 = other (on father's side)

DHQ

INTERVIEWER ☛ **(Fill out days of week 1st)**	_____	_____	_____	_____
Please indicate (circle) your experience of the following for each of the past three days.	**Today**	**Yesterday**	**2 days ago**	**3 days ago**
Allergy problems	YES NO	YES NO	YES NO	YES NO
Cold, flu, virus	YES NO	YES NO	YES NO	YES NO
Skin problem, rash	YES NO	YES NO	YES NO	YES NO
Urinary/vaginal infection	YES NO	YES NO	YES NO	YES NO
Mouth/lip sore	YES NO	YES NO	YES NO	YES NO
Did you take any drugs/medicine?	YES NO	YES NO	YES NO	YES NO
If Yes, what did you take? 1. 2. 3. 4. 5.	 _____ _____ _____ _____ _____	 _____ _____ _____ _____ _____	 _____ _____ _____ _____ _____	 _____ _____ _____ _____ _____
Did you have thoughts about breast cancer when you didn't mean to?	YES NO	YES NO	YES NO	YES NO

How many cigarettes, etc., did you smoke? **(indicate number in boxes)** ☛				
How many cups of coffee, or servings (8-12 ounces) of other caffeinated drinks (cola, tea, etc.) did you drink? ☛				
How many servings of alcoholic beverages (glasses of wine, bottles of beer, shots of liquor) did you drink? ☛				

How many hours of sleep did you get? ☛		Last night ____ hrs. ____ min.	2 nights ago ____ hrs. ____ min.	3 nights ago ____ hrs. ____ min.

POMS - Short Version

Below is a list of words that describe feelings people have. Please read each word carefully. Then CIRCLE ONE number which best describes HOW YOU HAVE BEEN FEELING **OVER THE LAST 24 HOURS.**

The numbers refer to these phrases: **0 = Not at all**
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

1 Friendly	0 1 2 3 4	33 Resentful	0 1 2 3 4
2 Tense	0 1 2 3 4	34 Nervous	0 1 2 3 4
3 Angry	0 1 2 3 4	36 Miserable	0 1 2 3 4
4 Worn out	0 1 2 3 4	38 Cheerful	0 1 2 3 4
5 Unhappy	0 1 2 3 4	39 Bitter	0 1 2 3 4
7 Lively	0 1 2 3 4	40 Exhausted	0 1 2 3 4
8 Confused	0 1 2 3 4	41 Anxious	0 1 2 3 4
12 Peeved	0 1 2 3 4	43 Good-natured	0 1 2 3 4
13 Considerate	0 1 2 3 4	48 Helpless	0 1 2 3 4
14 Sad	0 1 2 3 4	49 Weary	0 1 2 3 4
15 Active	0 1 2 3 4	50 Bewildered	0 1 2 3 4
16 On edge	0 1 2 3 4	51 Alert	0 1 2 3 4
17 Grouchy	0 1 2 3 4	52 Deceived	0 1 2 3 4
18 Blue	0 1 2 3 4	53 Furious	0 1 2 3 4
19 Energetic	0 1 2 3 4	55 Trusting	0 1 2 3 4
20 Panicky	0 1 2 3 4	56 Full of pep	0 1 2 3 4
21 Hopeless	0 1 2 3 4	58 Worthless	0 1 2 3 4
26 Uneasy	0 1 2 3 4	59 Forgetful	0 1 2 3 4
27 Restless	0 1 2 3 4	60 Carefree	0 1 2 3 4
28 Unable to concentrate	0 1 2 3 4	61 Terrified	0 1 2 3 4
29 Fatigued	0 1 2 3 4	63 Vigorous	0 1 2 3 4
30 Helpful	0 1 2 3 4	64 Uncertain about things	0 1 2 3 4
31 Annoyed	0 1 2 3 4	65 Bushed	0 1 2 3 4
32 Discouraged	0 1 2 3 4		

BSI

Below is a list of problems and complaints that people sometimes have. Read each item carefully, and select one of the numbered descriptors that best describes **HOW MUCH DISCOMFORT THAT PROBLEM HAS CAUSED YOU IN THE PAST MONTH, INCLUDING TODAY.** Please circle the number to the right of the problem. Do not skip any items. If you change your mind, erase your first circle completely.

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Nervousness or shakiness inside	1	2	3	4	5
2. Faintness or dizziness	1	2	3	4	5
3. The idea that someone else can control your thoughts	1	2	3	4	5
4. Feeling others are to blame for most of your troubles	1	2	3	4	5
5. Trouble remembering things	1	2	3	4	5
6. Feeling easily annoyed or irritated	1	2	3	4	5
7. Pains in heart or chest	1	2	3	4	5
8. Feeling afraid in open spaces	1	2	3	4	5
9. Thoughts of ending your life	1	2	3	4	5
10. Feeling that most people cannot be trusted	1	2	3	4	5
11. Poor appetite	1	2	3	4	5
12. Suddenly scared for no reason	1	2	3	4	5
13. Temper outbursts that you could not control	1	2	3	4	5
14. Feeling lonely even when you are with people	1	2	3	4	5
15. Feeling blocked in getting things done	1	2	3	4	5
16. Feeling lonely	1	2	3	4	5
17. Feeling blue	1	2	3	4	5
18. Feeling no interest in things	1	2	3	4	5
19. Feeling fearful	1	2	3	4	5
20. Your feelings being easily hurt	1	2	3	4	5
21. Feeling that people are unfriendly or dislike you.	1	2	3	4	5
22. Feeling inferior to others	1	2	3	4	5
23. Nausea or upset stomach	1	2	3	4	5
24. Feeling that you are watched or talked about by others	1	2	3	4	5

	Not at all	A little bit	Moderately	Quite a bit	Extremely
25. Trouble falling asleep	1	2	3	4	5
26. Having to check and double check what you do	1	2	3	4	5
27. Difficulty making decisions	1	2	3	4	5
28. Feeling afraid to travel on buses, subways, or train	1	2	3	4	5
29. Trouble getting (catching) your breath	1	2	3	4	5
30. Hot or cold spells (flashes)	1	2	3	4	5
31. Having to avoid certain things, places, or activities because they frightened you	1	2	3	4	5
32. Your mind going blank	1	2	3	4	5
33. Numbness or tingling in parts of your body	1	2	3	4	5
34. The idea that you should be punished for your sins	1	2	3	4	5
35. Feeling hopeless about the future	1	2	3	4	5
36. Trouble concentrating	1	2	3	4	5
37. Feeling weak in parts of your body	1	2	3	4	5
38. Feeling tense or keyed up	1	2	3	4	5
39. Thoughts of death or dying	1	2	3	4	5
40. Having urges to beat, injure, or harm someone	1	2	3	4	5
41. Having urges to break or smash things	1	2	3	4	5
42. Feeling very self-conscious with others	1	2	3	4	5
43. Feeling uneasy in crowds	1	2	3	4	5
44. Never feeling close to another person	1	2	3	4	5
45. Spells of terror or panic	1	2	3	4	5
46. Getting into frequent arguments	1	2	3	4	5
47. Feeling nervous when you are left alone	1	2	3	4	5
48. Others not giving you proper credit for your achievements	1	2	3	4	5
49. Feeling so restless that you couldn't sit still	1	2	3	4	5
50. Feelings of worthlessness	1	2	3	4	5
51. Feeling that people will take advantage of you if you let them	1	2	3	4	5

	Not at all	A little bit	Moderately	Quite a bit	Extremely
52. Feelings of guilt	1	2	3	4	5
53. The idea that something is wrong with your mind	1	2	3	4	5

Below is a list of comments made by people about stressful events.
IN THE LAST WEEK, INCLUDING TODAY, PLEASE INDICATE HOW FREQUENTLY THESE COMMENTS WERE TRUE FOR YOU ABOUT BREAST CANCER.
If the item did not occur, please mark the "not at all" column.

The numbers refer to these phrases:

0= Not at all
1= Rarely
3= Sometimes
5= Often

1.	Thought about it when I didn't mean to	0	1	3	5
2.	I avoided letting myself get upset when I thought about it or was reminded of it	0	1	3	5
3.	I tried to remove it from memory	0	1	3	5
4.	I had trouble falling asleep or staying asleep, because of pictures or thoughts about it that came into my mind	0	1	3	5
5.	I had waves of strong feelings about it	0	1	3	5
6.	I had dreams about it	0	1	3	5
7.	I stayed away from reminders of it	0	1	3	5
8.	I felt as if it was unreal	0	1	3	5
9.	I tried not to talk about it	0	1	3	5
10.	Pictures about it popped into my mind	0	1	3	5
11.	Other things kept making me think about it	0	1	3	5
12.	I was aware that I had a lot of feelings about it, but I didn't deal with them	0	1	3	5
13.	I tried not to think about it	0	1	3	5
14.	Any reminder brought back feelings about it	0	1	3	5
15.	My feelings about it were kind of numb	0	1	3	5
**	Have these experiences (#1-15, above) interfered with your daily activities?	0	1	3	5

Table 1: Descriptive Statistics for IES intrusions and avoidance subscales and their natural log transformations (+1) among premenopausal women with and without family histories of breast cancer

Descriptive Statistics									
	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
IES INTRUSION	183	.00	29.00	4.3661	6.22052	1.892	.180	3.361	.357
IES AVOIDANCE	182	.00	34.00	4.9615	7.03139	1.764	.180	2.954	.358
natural log of intrusion (+1)	183	.00	3.40	1.1055	1.06162	.426	.180	-1.135	.357
natural log of avoidance (+1)	182	.00	3.56	1.1353	1.14694	.395	.180	-1.318	.358

Table 2: Descriptive Statistics for IES intrusions and avoidance subscales and their natural log transformations (+1) among premenopausal women without first-degree relatives with breast cancer (no familial risk)

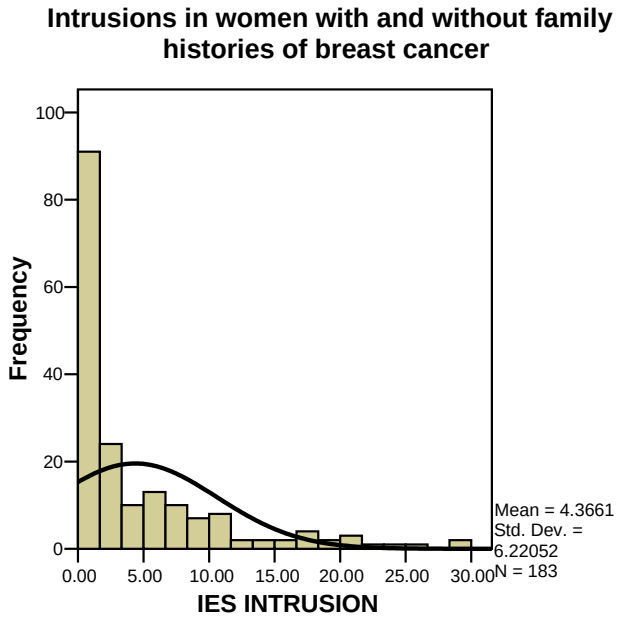
Descriptive Statistics(a)									
	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
IES INTRUSION	120	.00	29.00	3.7000	6.29339	2.349	.221	5.353	.438
IES AVOIDANCE	120	.00	32.00	3.7417	6.26380	2.300	.221	5.512	.438
natural log of intrusion (+1)	120	.00	3.40	.9324	1.03932	.772	.221	-.601	.438
natural log of avoidance (+1)	120	.00	3.50	.9214	1.06801	.732	.221	-.807	.438
NoFR									

Table 3: Descriptive Statistics for IES intrusions and avoidance subscales and their natural log transformations (+1) among premenopausal women with first-degree relatives diagnosed with breast cancer at postmenopausal age (low familial risk)

Descriptive Statistics(a)									
	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
IES INTRUSION	26	.00	20.00	3.2692	5.16572	2.226	.456	4.915	.887
IES AVOIDANCE	26	.00	26.00	6.8462	7.50835	.829	.456	-.243	.887
natural log of intrusion (+1)	26	.00	3.04	.9274	.99374	.671	.456	-.748	.887
natural log of avoidance (+1)	26	.00	3.30	1.4430	1.23940	-.057	.456	-1.753	.887
LoFR									

Table 4: Descriptive Statistics for IES intrusions and avoidance subscales and their natural log transformations (+1) among premenopausal women with first-degree relatives diagnosed with breast cancer at premenopausal age (high familial risk)

Descriptive Statistics(a)									
	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
IES INTRUSION	37	.00	23.00	7.2973	5.91545	.855	.388	.370	.759
IES AVOIDANCE	36	.00	34.00	7.6667	8.16963	1.390	.393	1.983	.768
natural log of intrusion (+1)	37	.00	3.18	1.7922	.91320	-.690	.388	-.450	.759
natural log of avoidance (+1)	36	.00	3.56	1.6258	1.16309	-.327	.393	-1.233	.768
HiFR									



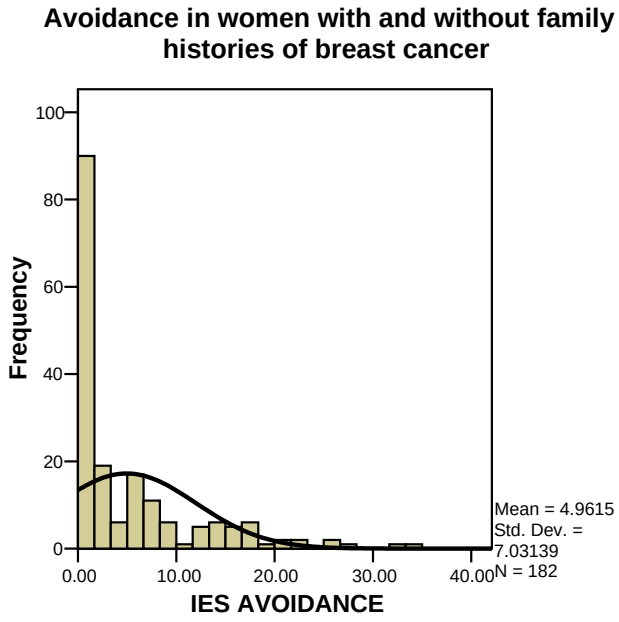
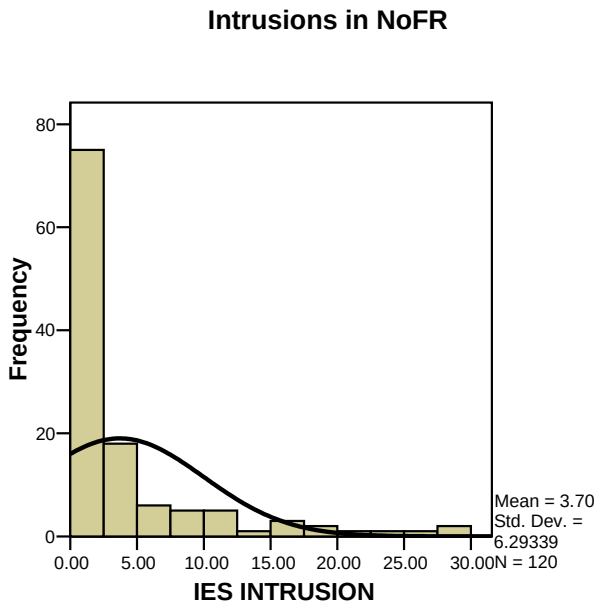


Figure 1. IES Intrusion and Avoidance Subscales among premenopausal women with and without family histories of breast cancer: Frequencies on raw scores



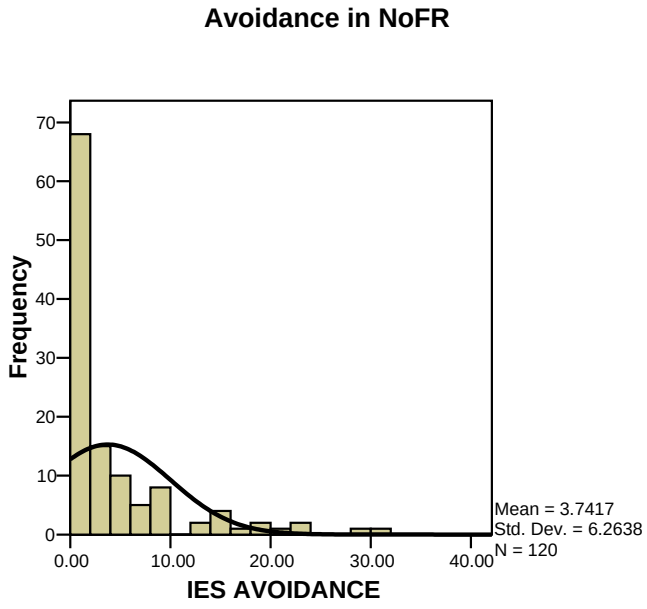
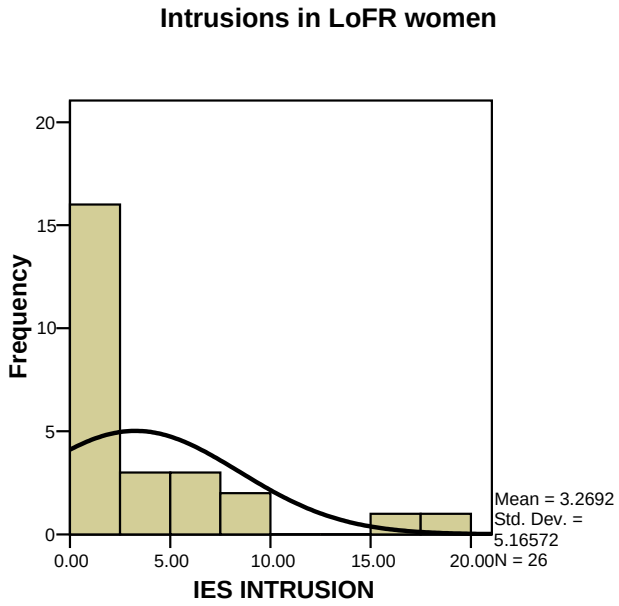


Figure 2. IES Intrusion and Avoidance Subscales among premenopausal women without first-degree relatives with breast cancer (no familial risk): Frequencies on raw scores



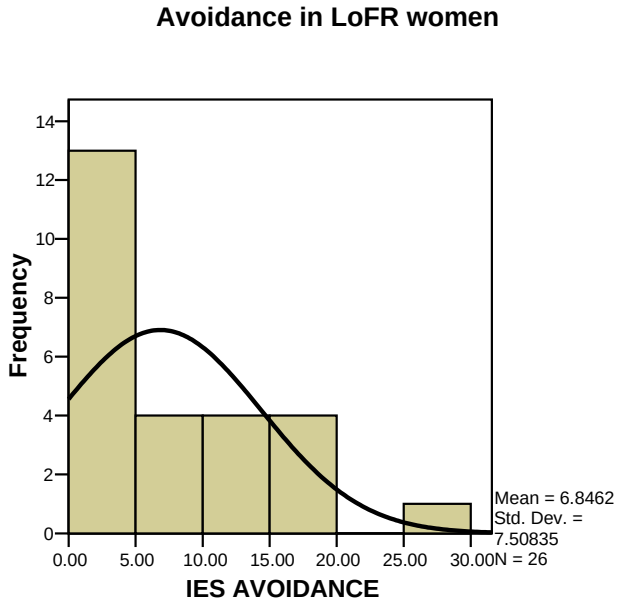


Figure 3. IES Intrusion and Avoidance Subscales among premenopausal women with first-degree relatives diagnosed with breast cancer at postmenopausal age (low familial risk): Frequencies on raw scores

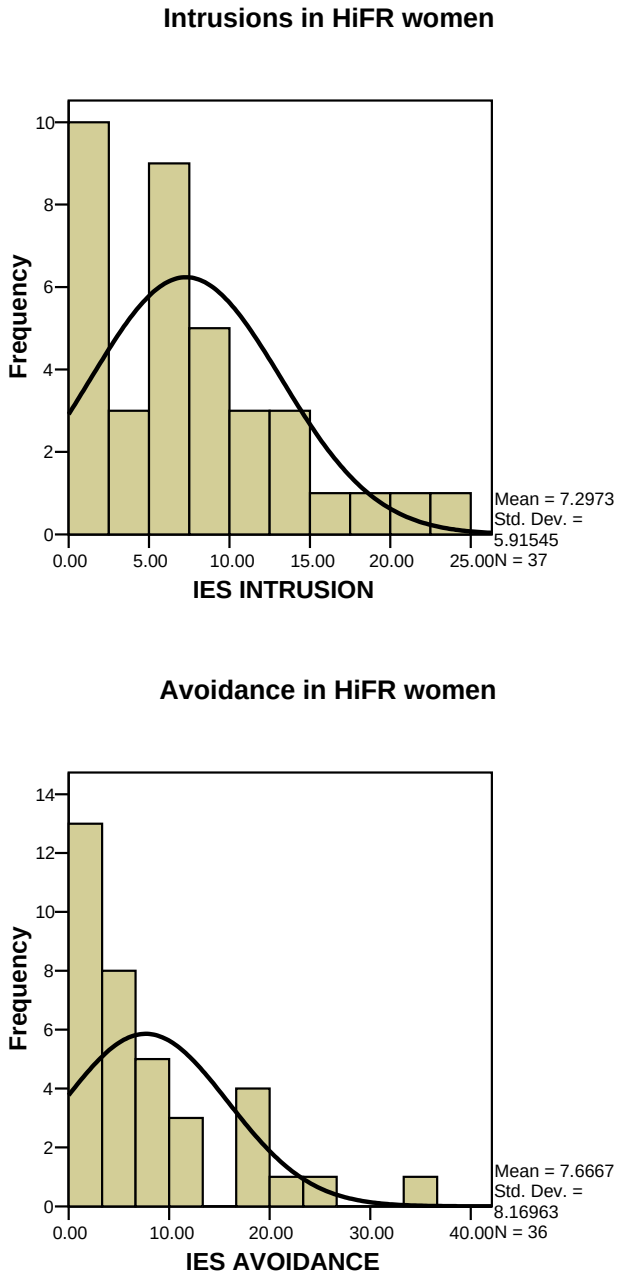


Figure 4. IES Intrusion and Avoidance Subscales among premenopausal women with first-degree relatives diagnosed with breast cancer at premenopausal age (high familial risk): Frequencies on raw scores

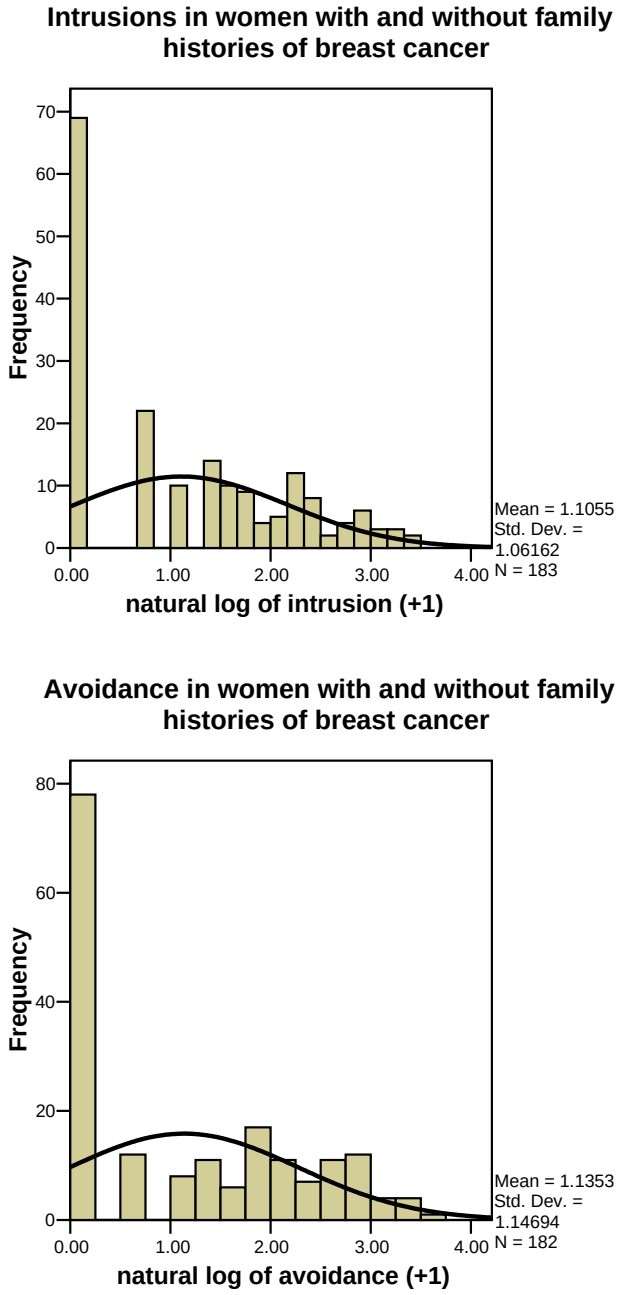


Figure 5. IES Intrusion and Avoidance Subscales among premenopausal women with and without family histories of breast cancer: Frequencies on natural log-transformed scores (+1)

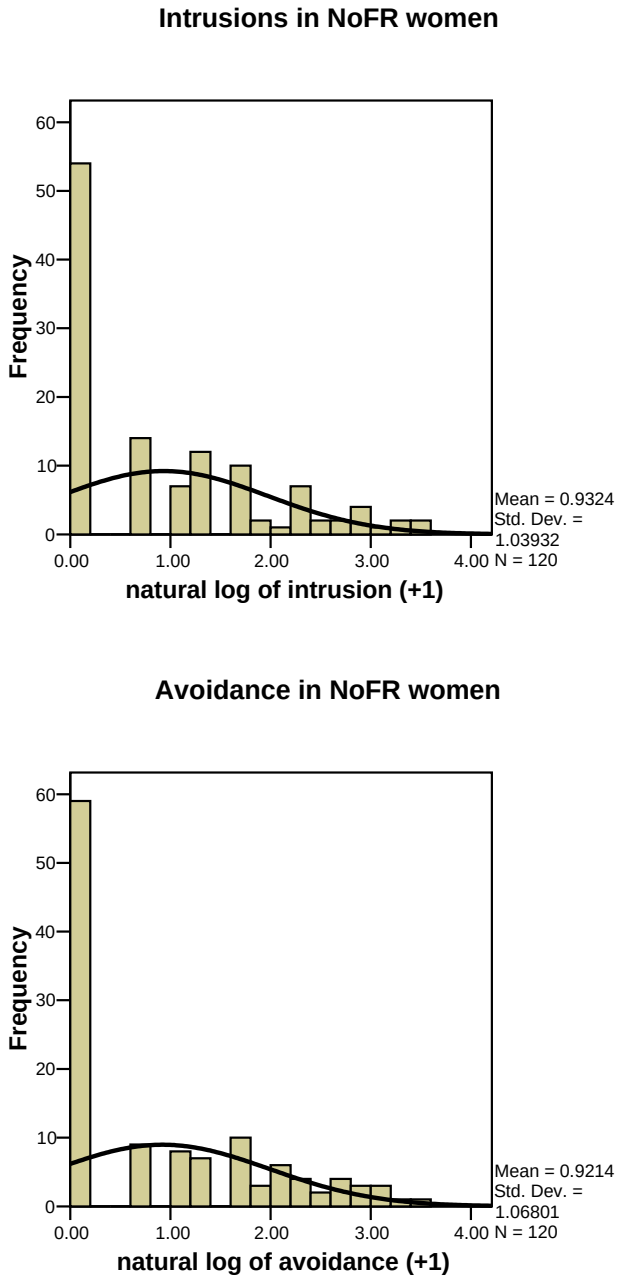


Figure 6. IES Intrusion and Avoidance Subscales among premenopausal women without first-degree relatives with breast cancer (no familial risk): Frequencies on natural log-transformed scores (+1)

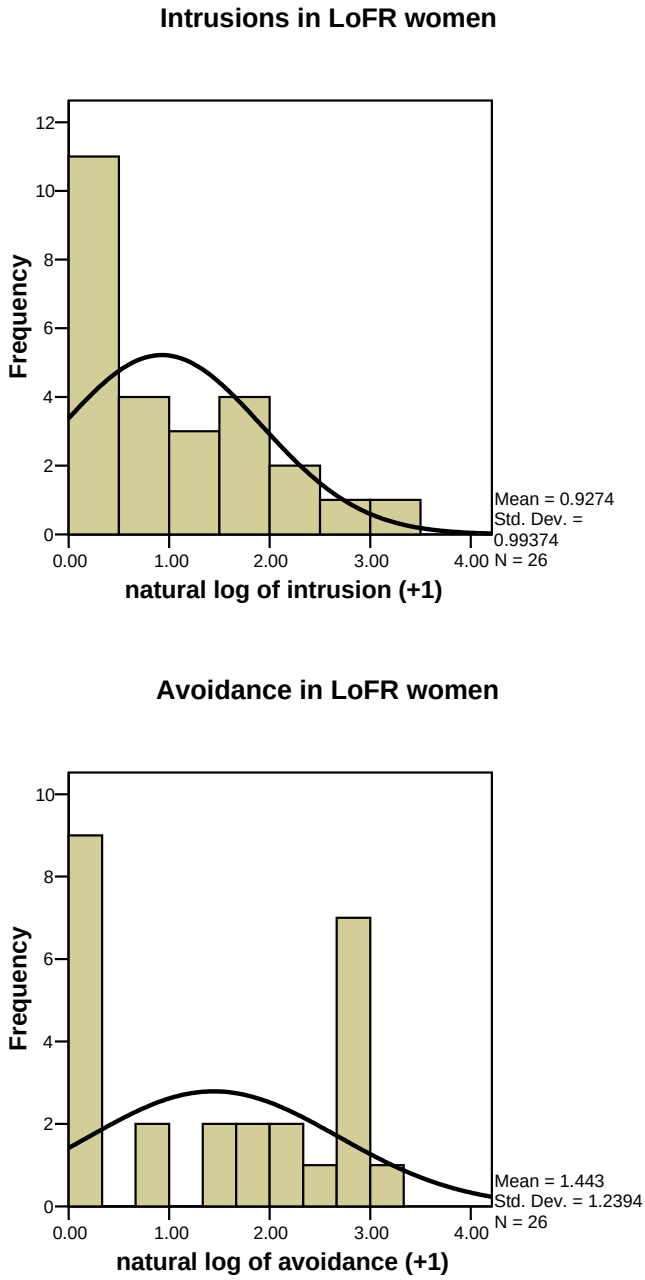


Figure 7. IES Intrusion and Avoidance Subscales among premenopausal women with first-degree relatives diagnosed with breast cancer at postmenopausal age (low familial risk): Frequencies on natural log-transformed scores (+1)

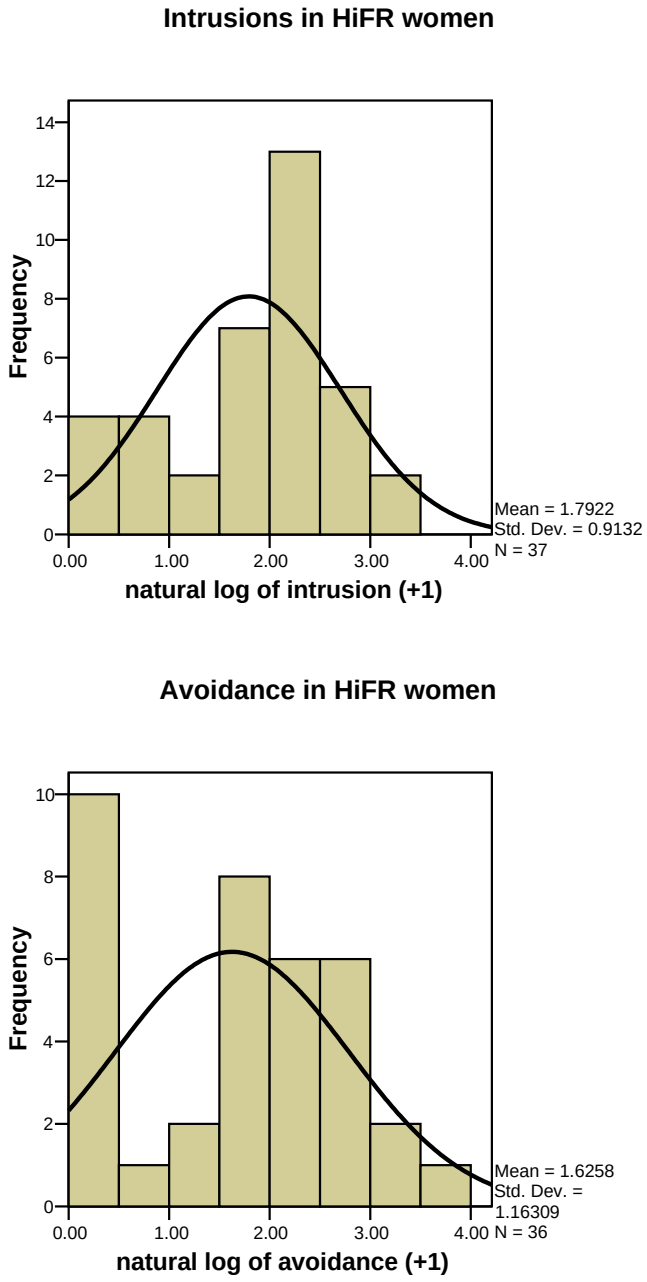


Figure 8. IES Intrusion and Avoidance Subscales among premenopausal women with first-degree relatives diagnosed with breast cancer at premenopausal age (high familial risk): Frequencies on natural log-transformed scores (+1)

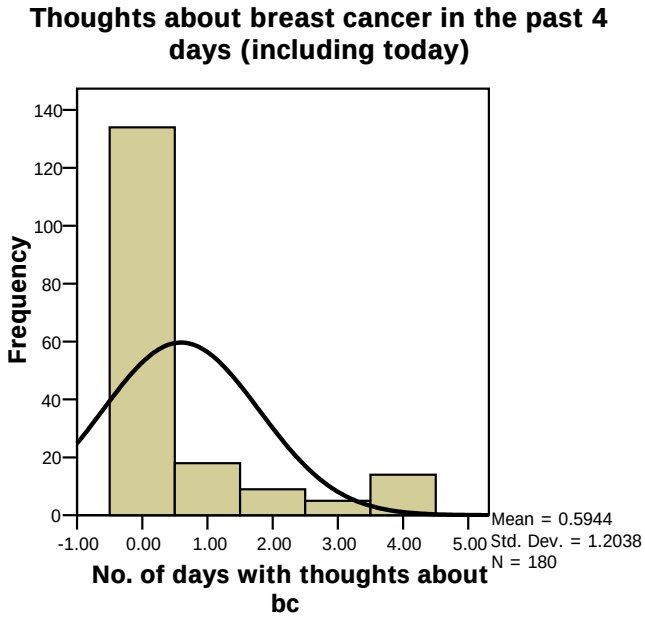


Figure 1. Number of days with thoughts about breast cancer in premenopausal women with and without family histories of breast cancer.

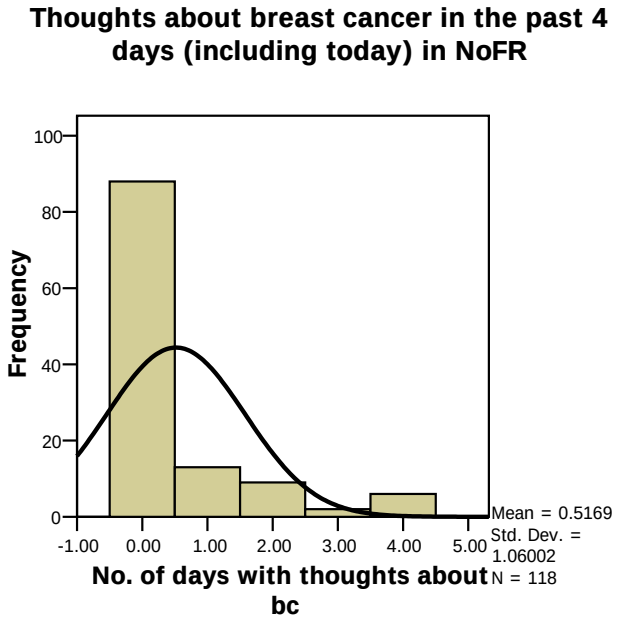


Figure 2. Number of days with thoughts about breast cancer in premenopausal women without first-degree relatives with breast cancer (no familial risk).

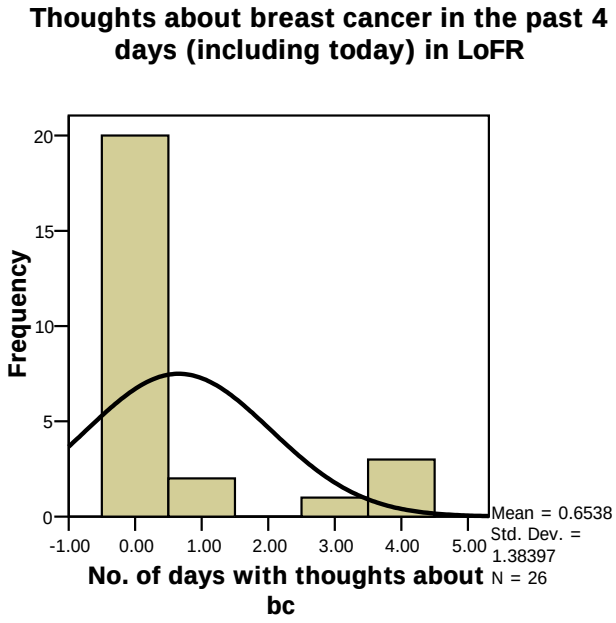


Figure 3. Number of days with thoughts about breast cancer in premenopausal women with first-degree relatives with breast cancer diagnosed at postmenopausal age (low familial risk).

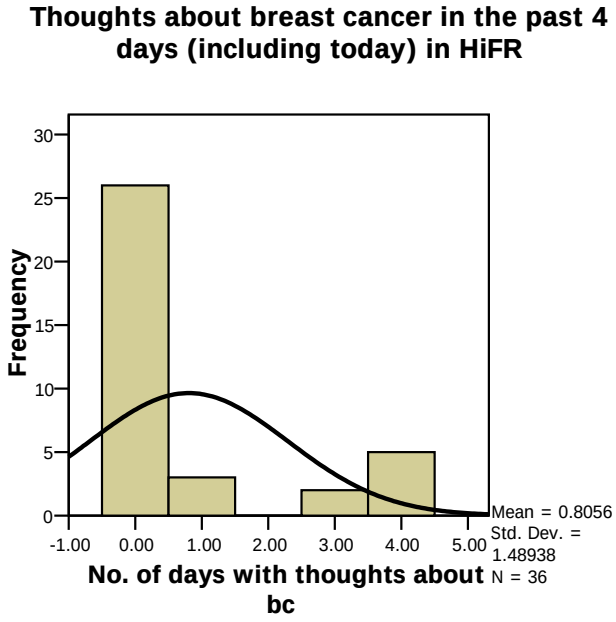


Figure 4. Number of days with thoughts about breast cancer in premenopausal women with first-degree relatives with breast cancer diagnosed at premenopausal age (high familial risk).

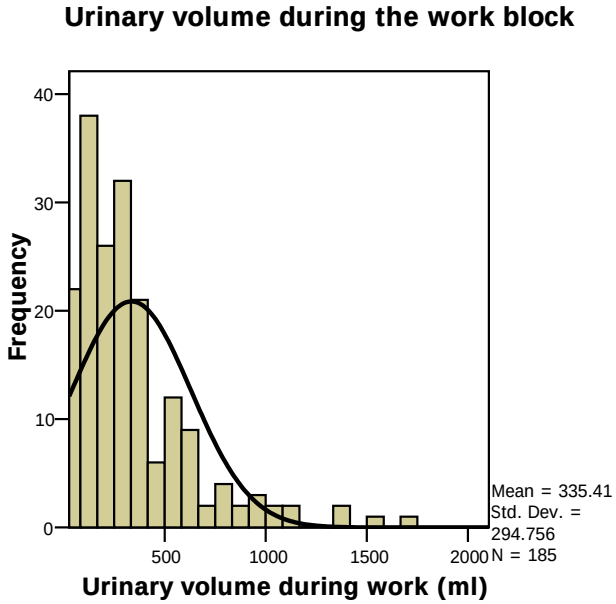


Figure 1. Urinary volume during the work block in premenopausal women with and without family histories of breast cancer

Table 1: Univariate Analysis comparing urinary volumes at work across the three groups (NoFR/LoFR/HiFR)

Between-Subjects Factors

		Value Label	N
Familial Risk Groups	.00	NoFR	121
	1.00	LoFR	27
	2.00	HiFR	37

Descriptive Statistics
Dependent Variable: urinary volume at work

Familial Risk Group	Mean	Std. Deviation	N
NoFR	306.09	288.414	121
LoFR	454.59	363.519	27
HiFR	344.32	240.253	37
Total	335.41	294.756	185

Levene's Test of Equality of Error Variances(a) Dependent Variable: urinary volume at work			
F	**df1**	**df2**	**Sig.**
2.168	2	182	.117

Tests of Between-Subjects Effects Dependent Variable: urinary volume at work					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	490474.152(a)	2	245237.076	2.880	.059
Intercept	16881890.843	1	16881890.843	198.281	.000
Familial Risk Group	490474.152	2	245237.076	2.880	.059
Error	15495686.627	182	85141.135		
Total	36798737.000	185			
Corrected Total	15986160.778	184			
a R Squared = .031 (Adjusted R Squared = .020)					

Contrast Results (K Matrix)			
Familial Risk Groups Simple Contrast(a)			**Dependent Variable**
			urinary volume at work
LoFR vs. NoFR	**Contrast Estimate**		148.502
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		148.502
	Std. Error		62.105
	Sig.		.018
	95% Confidence Interval for Difference	**Lower Bound**	25.963
		Upper Bound	271.040
HiFR vs. NoFR	**Contrast Estimate**		38.233
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		38.233
	Std. Error		54.816
	Sig.		.486
	95% Confidence Interval for Difference	**Lower Bound**	-69.923
		Upper Bound	146.389

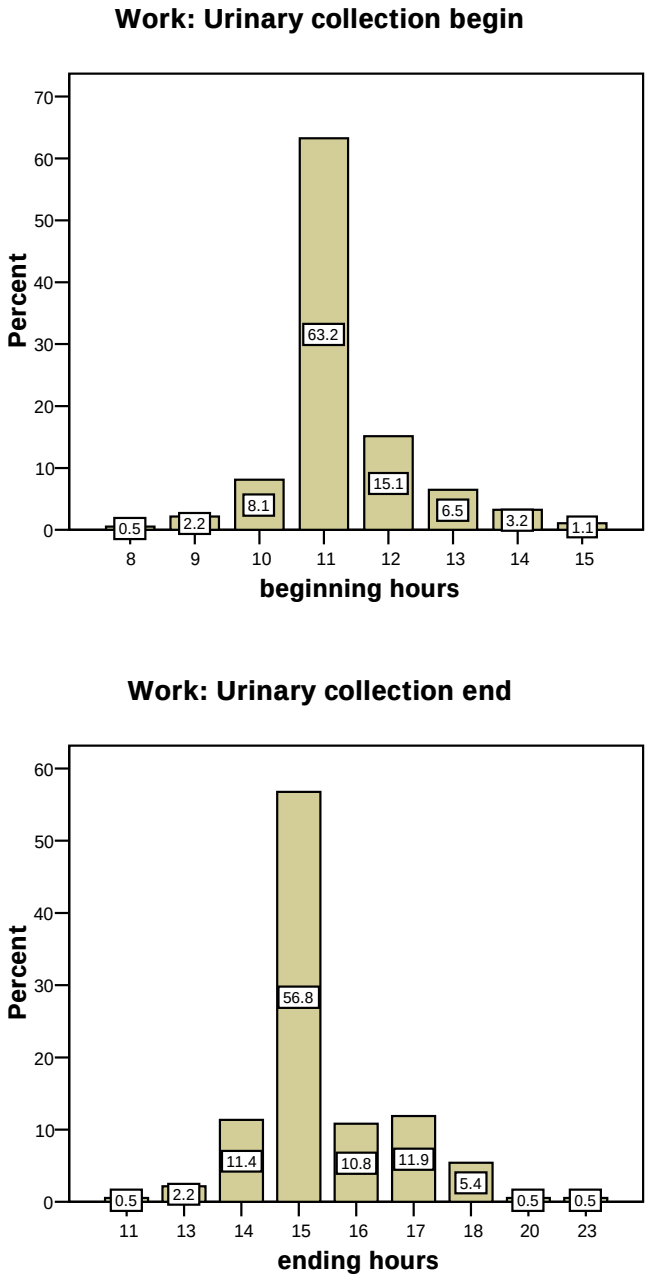


Figure 2. Percentages for urinary collection times (beginning and end) during the work block

Tabelle 2: Univariate analysis comparing groups on collection times (beginning and end)

Between-Subjects Factors			
		Value Label	**N**
Familial Risk Groups	**.00**	NoFR	121
	1.00	LoFR	27
	2.00	HiFR	37

Tests of Between-Subjects Effects Dependent Variable: work beginning hour					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1.088(a)	2	.544	.532	.589
Intercept	15982.792	1	15982.792	15614.275	.000
prepost	1.088	2	.544	.532	.589
Error	186.295	182	1.024		
Total	23731.000	185			
Corrected Total	187.384	184			
a R Squared = .006 (Adjusted R Squared = -.005)					

Between-Subjects Factors			
		Value Label	**N**
Familial Risk Groups	**.00**	NoFR	121
	1.00	LoFR	27
	2.00	HiFR	37

Tests of Between-Subjects Effects Dependent Variable: work ending hour					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	.413(a)	2	.206	.121	.886
Intercept	29520.888	1	29520.888	17332.342	.000
prepost	.413	2	.206	.121	.886
Error	309.987	182	1.703		
Total	44185.000	185			
Corrected Total	310.400	184			
a R Squared = .001 (Adjusted R Squared = -.010)					

Table 3: Univariate analysis comparing collection duration for the work block among groups (NoFR/LoFR/HiFR)

Between-Subjects Factors			
		Value Label	**N**
Familial Risk Groups	**.00**	NoFR	121
	1.00	LoFR	26
	2.00	HiFR	37

Descriptive Statistics Dependent Variable: work period (in hrs)			
Familial Risk Groups	**Mean**	**Std. Deviation**	**N**
NoFR	4.1719	1.05435	121
LoFR	4.0763	1.09988	26
HiFR	4.2207	.99676	37
Total	4.1682	1.04471	184

Levene's Test of Equality of Error Variances(a) Dependent Variable: work period (in hrs)			
F	**df1**	**df2**	**Sig.**
.039	2	181	.962

Tests of Between-Subjects Effects Dependent Variable: work period (in hrs)					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	.323(a)	2	.162	.147	.864
Intercept	2108.029	1	2108.029	1913.429	.000
prepost	.323	2	.162	.147	.864
Error	199.408	181	1.102		
Total	3396.538	184			
Corrected Total	199.732	183			
a R Squared = .002 (Adjusted R Squared = -.009)					

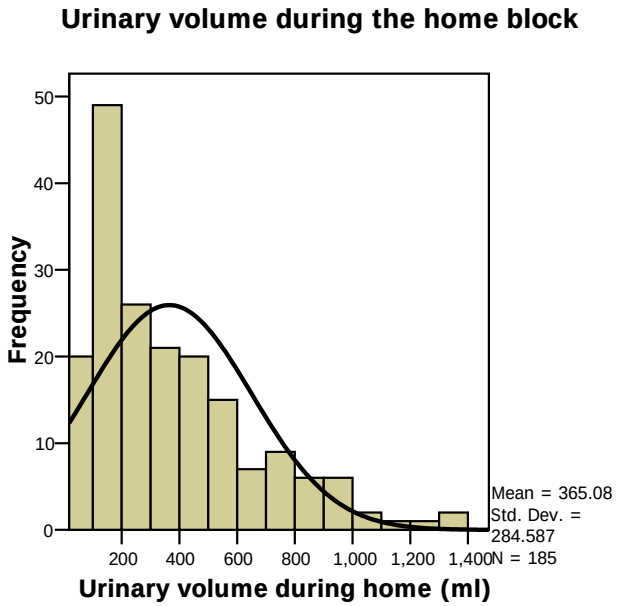


Figure 3. Urinary volume during the home block in premenopausal women with and without family histories of breast cancer

Table 4: Univariate Analysis comparing urinary volumes at home across the three groups (NoFR/LoFR/HiFR)

Between-Subjects Factors			
		Value Label	**N**
Familial Risk Groups	**.00**	NoFR	121
	1.00	LoFR	27
	2.00	HiFR	37

Descriptive Statistics Dependent Variable: urinary volume at home			
first-degree hx of pre/postmenopausal bc	**Mean**	**Std. Deviation**	**N**
NoFR	343.04	273.593	121
LoFR	473.33	328.068	27
HiFR	358.16	276.104	37
Total	365.08	284.587	185

Levene's Test of Equality of Error Variances(a) Dependent Variable: urinary volume at home			
F	**df1**	**df2**	**Sig.**
.306	2	182	.737

Tests of Between-Subjects Effects
Dependent Variable: urinary volume at home

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	376947.963(a)	2	188473.982	2.362	.097
Intercept	19073203.838	1	19073203.838	238.988	.000
Familial Risk Group	376947.963	2	188473.982	2.362	.097
Error	14525117.820	182	79808.340		
Total	39559642.000	185			
Corrected Total	14902065.784	184			

a R Squared = .025 (Adjusted R Squared = .015)

Contrast Results (K Matrix)

Familial Risk Group Simple Contrast(a)			Dependent Variable
			urinary volume at home
LoFR vs. NoFR	**Contrast Estimate**		130.292
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		130.292
	Std. Error		60.129
	Sig.		.032
	95% Confidence Interval for Difference	**Lower Bound**	11.653
		Upper Bound	248.931
HiFR vs. LoFR	**Contrast Estimate**		15.121
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		15.121
	Std. Error		53.071
	Sig.		.776
	95% Confidence Interval for Difference	**Lower Bound**	-89.593
		Upper Bound	119.835

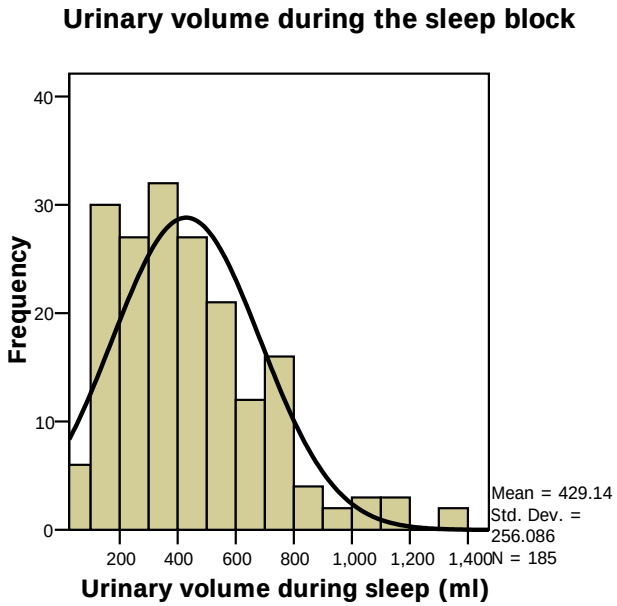


Figure 4. Urinary volume during the sleep block in premenopausal women with and without family histories of breast cancer

Table 5: Univariate Analysis comparing urinary volumes during sleep across the three groups (NoFR/LoFR/HiFR)

Between-Subjects Factors

		Value Label	N
Familial Risk Groups	.00	NoFR	121
	1.00	LoFR	27
	2.00	HiFR	37

Descriptive Statistics
Dependent Variable: Urinary volume during sleep

Familial Risk Groups	Mean	Std. Deviation	N
NoFR	397.75	234.509	121
LoFR	512.44	343.329	27
HiFR	471.00	237.539	37
Total	429.14	256.086	185

Levene's Test of Equality of Error Variances(a)
Dependent Variable: Urinary volume during sleep

F	df1	df2	Sig.
4.386	2	182	.014

Tests of Between-Subjects Effects
Dependent Variable: Urinary volume during sleep

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	371413.117(a)	2	185706.559	2.890	.058
Intercept	26375537.886	1	26375537.886	410.449	.000
Familial Risk Group	371413.117	2	185706.559	2.890	.058
Error	11695351.229	182	64260.172		
Total	46136661.000	185			
Corrected Total	12066764.346	184			

a R Squared = .031 (Adjusted R Squared = .020)

Contrast Results (K Matrix)

Familial Risk Groups Simple Contrast(a)			Dependent Variable: Urinary volume during sleep
LoFR vs. NoFR	**Contrast Estimate**		114.692
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		114.692
	Std. Error		53.954
	Sig.		.035
	95% Confidence Interval for Difference	**Lower Bound**	8.236
		Upper Bound	221.149
HiFR vs. LoFR	**Contrast Estimate**		73.248
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		73.248
	Std. Error		47.622
	Sig.		.126
	95% Confidence Interval for Difference	**Lower Bound**	-20.714
		Upper Bound	167.210

Table 1: Descriptives for percentage of mood readings at work and at home

Descriptive Statistics						
	N	Range	Minimum	Maximum	Mean	Std. Deviation
no. recordings anxious at work x 100/total no. recordings work	116	85.71	.00	85.71	10.0370	16.37787
no. recordings anxious at home x 100/total no. recordings home	116	71.43	.00	71.43	2.9427	9.60158
no. recordings angry at work x 100/total no. recordings work	116	50.00	.00	50.00	3.2505	8.74996
no. recordings angry at home x 100/total no. recordings home	116	71.43	.00	71.43	3.0608	9.61613
no. recordings sad at work x 100/total no. recordings work	116	46.15	.00	46.15	1.4026	5.67348
no. recordings sad at home x 100/total no. recordings home	116	71.43	.00	71.43	1.3917	7.56188
no. recordings happy at work x 100/total no. recordings work	116	100.00	.00	100.00	17.0504	28.31313
no. recordings happy at home x 100/total no. recordings home	116	100.00	.00	100.00	17.8799	31.35379

Table 2: One-Sample t-test comparing mean percentage of anxious readings at home with mean percentage anxious reading at work (Mean=10.04, STD=16.38)

One-Sample Test						
	Test Value = 10					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
no. recordings anxious at home x 100/total no. recordings home	-7.916	115	.000	-7.05731	-8.8232	-5.2914

Table 3: Univariate Analysis comparing mean percentage of anxious readings at work between NoFR (n=78), LoFR (n=17), and HiFR (n=21)

Descriptive Statistics
Dependent Variable: no. recordings anxious at work x 100/total no. recordings work

Familial Risk Groups	Mean	Std. Deviation	N
NoFR	10.1921	17.86361	78
LoFR	14.8350	16.55538	17
HiFR	5.5769	7.34618	21
Total	10.0370	16.37787	116

Tests of Between-Subjects Effects
Dependent Variable: no. recordings anxious at work x 100/total no. recordings work

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	810.979(a)	2	405.490	1.526	.222
Intercept	7853.235	1	7853.235	29.545	.000
Familial Risk Group	810.979	2	405.490	1.526	.222
Error	30035.990	113	265.805		
Total	42532.934	116			
Corrected Total	30846.969	115			

a R Squared = .026 (Adjusted R Squared = .009)

Table 4: Chi-Square Test comparing no anxious readings at work with some anxious readings at work among familial risk groups

			Familial Risk Groups			Total
			NoFR	LoFR	HiFR	
Anxious At Work	**No**	**Count**	41	7	11	59
		% within anxious at work	69.5%	11.9%	18.6%	100.0%
		% within Familial Risk Group	52.6%	41.2%	52.4%	50.9%
		% of Total	35.3%	6.0%	9.5%	50.9%
		Std. Residual	.2	-.6	.1	
	Yes	**Count**	37	10	10	57
		% within anxious at work	64.9%	17.5%	17.5%	100.0%
		% within Familial Risk Group	47.4%	58.8%	47.6%	49.1%
		% of Total	31.9%	8.6%	8.6%	49.1%
		Std. Residual	-.2	.6	-.1	

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.748(a)	2	.688
Likelihood Ratio	.751	2	.687
Linear-by-Linear Association	.057	1	.812
N of Valid Cases	116		
a 0 cells (.0%) have expected count less than 5. The minimum expected count is 8.35.			

Table 5: Spearman's Rho Rank correlations between percentage of anxious readings at work and work cortisol levels

Correlations				
			no. recordings anxious at work x 100/total no. recordings work	**Work Cortisol**
Spearman's rho	**no. recordings anxious at work x 100/total no. recordings work**	**Correlation Coefficient**	1.000	-.007
		Sig. (2-tailed)	.	.937
		N	116	116