ACUTE ENDOCRINE STRESS REACIVITY AND RECOVERY IN WOMEN AT FAMILIAL RISK OF BREAST CANCER:

ASSOCIATION WITH PERCEIVED STRESS AND DEPRESSIVE SYMPTOMS

Dissertation
zur Erlangung der Würde des Doktors der Philosophie
der Universität Hamburg

vorgelegt von

Stefan M. Gold

aus Karlsruhe

Hamburg, 2003

Referent: Prof. Dr. Dr. Uwe Koch

Co-Referent: Prof. Dr. Dana H. Bovbjerg

Datum der letzten mündlichen Prüfung: 12. Mai 2003

For my parents
and Christina.

Acknowledgements:
I am deeply indepted to Dr. Dana H. Bovbjerg (Mt. Sinai School of Medicine), who invited me to New York and made this project possible. It has been a privilige to interact intellectually and scientifically with him during the 2 years I had the pleasure to work in New York City. I owe a great dept of gratitude to all the people who worked with me at Mt. Sinai: Heiddis Valdimarsdottir, Jan Jeremias, Janae Ostolaza, Maria Avellino, Vicki LaVista and everyone else at the Ruttenberg Cancer Center. I would also like to thank Sandra Zakowski (Finch University Chicago). I am especially grateful to PD Dr. Dr. Karl-Heinz Schulz and Dr. Christoph Heesen (University Hospital Hamburg-Eppendorf) for the help, support and advice I have received over the course of the past years. I thank Dr. Holger Schulz and Prof. Dr. Dr. Uwe Koch for their continued support. I am grateful to my brother for dicussing relevant and irrelevant literature. I would like to thank Dania Hückmann for critically reviewing the manuscript and for flying Singapore Airlines. Finally, thanks to Carola for supporting me, especially during the final phase of the project. And to Jenny, for encouraging me to begin (and in a way to complete) this work.

TABLE OF CONTENTS **PAGE**

1. INTRODUCTION

Cancer risk, stress, and neuroendocrine regulation

The notion that cancer is not purely a somatic process but may be mediated by psychologic factors has ancient origins, beginning in the second century. The Greek physician Galen claimed that women of "melancholic" temperament were more likely to develop breast cancer. Over the course of the centuries, physicians reported that cancer patients are more frequently exhibiting fear, anxiety, hopelessness and depression (see Greer, 1983 for a collection of historical examples). Various reports of emotional distress preceding the diagnosis of cancer can be found in the literature from the 18th and 19th century (see Kowal, 1955). The interest in psychosocial precursors declined in the first half of the 20th century, when medical research was again dominated by Descartes' views (1596-1650). The French philosopher had considered the mind as a distinctly separated entity ("res cogitans") which was independent from the mechanistic body ("res extensa") (see Cottingham, 1988). In the early 20th century, Greer (1983) mentions only one psychodynamic case study which investigated the association between emotion and cancer (Evans, 1924). A revival of interest began in 1948, when Miller and Jones reported six cancer cases, who showed "marked emotional stress" before leukemia had been diagnosed. A number descriptive studies followed in the 1950s and 1960s, which reported that the appearance of cancer was frequently preceded by personal losses (see Greer, 1983 for examples).

Beginning in the early 1970s, more details about how the brain, the endocrine, and the immune system interact became available. Ader and coworkers (e.g. Ader & Cohen, 1975; Ader et al., 1982) discovered that immune function could be classically conditioned. John Hadden (e.g. 1970) examined adrenergic receptors on lymphocytes and their relevance for the regulation of immune function. Hugo Besedovsky and colleagues could demonstrate that the nervous system perceives and responds to signals emitted by the immune system (e.g. 1977). This area of research for the first time offered hypotheses and pathways by which emotion could affect physiological parameters and influence the development of diseases such as cancer. A historical synopsis of how the field of psychoneuroimmunology evolved can be found in Sternberg (2001) or Ader (2000).

Clinical research has, however, often ignored the available evidence for these pathways and mechanisms and has yet to provide substantial evidence for the importance of psychosocial factors regarding cancer risk. In addition, research that attempted to link psychosocial

stressors with tumor development or progression has also faced many methodological difficulties. For example, stage of disease can have a profound effect on how patients feel, and cancer treatments such as chemotherapy and radiation are associated with a number of side-effects (Kiecolt-Glaser & Glaser, 1999). Research has yet to provide clear-cut evidence for the importance of psychological factors for cancer development and progression.

In a recent review, Dalton et al. (2002) state that to this day, there is no indication "that these [psychosocial] factors play a major role in cancer causation, when the possible roles of biases, confounding and chance are taken into account" (p.1321). Their paper reviews studies examining major life events, depression and depressive mood, as well as personality or personality traits in any form of cancer. Butow et al. (2000) come to a similar conclusion by analyzing the literature on life events, coping styles, social support, emotional and personality factors specifically in breast cancer. Both reviews highlight that methodological problems (e.g. selection of comparison groups, or lack of adjustment for other risk factors such as age or smoking) often make accurate data interpretation difficult if not impossible. Dalton et al. (2002) add that other plausible explanations for an association between psychosocial factors and cancer are often not considered in the interpretation of results. For example, the possibility of a common, underlying biological susceptibility is often ignored. However, in most cases, it cannot be ruled out that such a biological predisposition may determine personality, the response to stress, and the risk for developing cancer (p. 1322). Research, which examines the contribution of biological factors independently and in combination with psychological factors may therefore help to find more consistent results.

According to Butow and co-workers (2000), "a significant weakness in this area of research is the essentially atheoretical approach" (p.178). They strongly recommend to articulate the model that forms the basis of the research and to develop testable hypotheses. The authors assert that the "progression in the understanding of the role of psychosocial variables in breast cancer development and the mechanisms by which they exert their effects, requires the guidance of a model which acknowledges links with the endocrine, nervous, and immune systems" (p.178).

Recently, such models have become available to help the researchers develop theory-driven hypotheses and to propose potentially mediating pathways. They also allow the interpretation of effects with respect to a theoretical background. An overview of how evidence from the psychoneuroimmunology literature may be incorporated into clinical oncology research has been published by Bovbjerg et al. (1999).

Andersen et al. (1994) have proposed a biobehavioral model of cancer. This model takes psychological, neuroendocrine, immunological, behavioral (as health behaviors or compliance with treatment and / or screening), and biological factors into account. Most of the hypothesized associations between two given variables in the model have received some empirical support from molecular biology, psychology, oncology, or epidemiology. However, only very few studies have to date tested multi-factorial associations within the model (see Schulz & Gold, 1999).

One highly relevant area to investigate the effects of psychosocial factors are populations which are already at increased risk of developing a disease, e.g. those with a genetic susceptibility (Ader, 2000, p. 174). Andersen et al. (1994) have highlighted that stress-effects on endocrine and immune function may not be biologically relevant in people with no other additional risk factors. In contrast, they might have a severe impact on disease incidence in groups with an already heightened susceptibility. Several risk factors for breast cancer have been identified. These include age at menopause, age at menarche, and number of children. Having a family history of breast cancer, however, is the strongest predictor that a woman will develop the disease at one point during her life (Arver et al., 2000). Furthermore, breast cancer has been suggested as a relevant area for investigating the association between cancer and stress, since it is hormonally sensitive. Psychosocial factors which elicit endocrine responses may thus play a greater role in cancer of the breast than at other sites (Butow et al., 2000).

OVERVIEW

This study investigates the association between familial breast cancer risk, psychological distress, and acute endocrine stress responses. Based on the biobehavioral model of breast cancer risk (Bovbjerg & Valdimarsdottir, 2001) and the theory of allostatic load (McEwen, 1998), the study was designed to examine endocrine stress responses in women at increased familial breast cancer risk. Alterations of these responses were expected to be associated with increased levels of psychological distress hypothesized in this population.

In the **first section** of the theoretical portion, evidence for an increased breast cancer risk for women with a family history of this disease will be reviewed. Subsequently, I will outline a theoretical model of how having a family history of breast cancer affects risk perception, psychological well-being and health behaviors. Furthermore, literature on the prevalence of psychological distress in women at increased breast cancer risk is reviewed and discussed.

The biobehavioral model proposed by Bovbjerg & Valdimarsdottir (2001) is then introduced to illustrate the potential impact of psychological distress on cancer incidence. This model also considers health behaviors, endocrine pathways, and immune defense alterations found in women at familial breast cancer risk.

In the **second section**, theoretical stress concepts are presented. The anatomical and physiological fundamentals of the acute stress response are briefly reviewed. Furthermore, the kinetics of the acute stress response are summarized. Here, each of the body's different stress systems plays a time-sensitive role in this cascade of orchestrated responses. Subsequently, commonly used experimental human stress paradigms are outlined. Exposure to chronic stress has been found to affect acute endocrine stress responses in animals and humans. This literature will be described in the **third section**.

In the **fourth section**, Bruce McEwen's concept of allostasis and allostatic load (1998) is described. This model provides a theoretical and empirical background for illustrating potential long-term effects of altered acute endocrine stress responses. Finally, the research questions and hypotheses for the present study are derived.

After presentation of the **methods** and **results** of the study, findings will be discussed with reference to the empirical and theoretical background and **implications for future research** will be outlined.

2. THEORETICAL BACKGROUND

2.1. Familial risk of breast cancer

2.1.1. Cancer risk and family history

Breast cancer is the most common malignancy among women worldwide and its incidence is increasing (Parkin et al., 1999). It is currently estimated that in 2003, more than 211,300 women in the US will be diagnosed with breast cancer and that 39,800 will die of the disease. Breast cancer is the most common cause of cancer in American women and ranks second after lung cancer among cancer deaths in women (American Cancer Society, 2003). In Germany, breast cancer is the most commonly diagnosed malignancy in women and is the most frequent cause of cancer death (approx. 25%, Arbeitsgemeinschaft Bevölkerungsbezogener Krebsregister in Deutschland, 2002).

A genetic susceptibility for breast cancer has long been assumed in families with increased incidence of the disease. More than two-fold increases in risk for women with one first-degree relative diagnosed with breast cancer has been reported in a recent meta-analysis including 74 studies published between 1935 and 1995 (Pharoah et al., 1997). Women with two affected first-degree relatives are approximately 4 to 6 times more likely to develop the disease. The risk estimates are even higher if the relative was affected at an early age (see Bovbjerg & Valdimarsdottir, 2001). In the mid-1990s, two breast-cancer inducing gene mutations have successfully been sequenced and localized to chromosome 17 (BRCA1, Miki et al., 1994) and chromosome 13 (BRCA2, Wooster et al., 1995). Recent epidemiological studies have re-estimated the heredity of breast cancer between 5 and 10%. Not all of them, however, can be linked to the "risk genes" BRCA1 and BRCA2. Authors have estimated that these genes account 84% (Claus et al., 1996) or less (40-50%, see Bovbjerg & Valdimarsdottir, 2001) of the hereditary cancers. Bovbjerg and Valdimarsdottir (2001) highlight that the presence of the mutation may be important but not sufficient to account for the development of breast cancer. Hence, other genetic as well as non-genetic factors may be important. Health behaviors, endocrine and immune function are likely to be involved in the development of cancer (or lack thereof) in women with family histories. The following section describes theories and empirical evidence for a multi-factorial disease model.

2.1.2. Effects of familial risk on risk perception, distress, and health behaviors:

Rees and co-workers (2001) have proposed a theoretical model of how a woman's perceived risk of developing breast cancer is determined by experience with relatives suffering from the disease, and how this, in turn, may influence psychological distress and health behaviors. This model, which was based on the Tversky's and Kahnemann's heuristics and biases (1974), is briefly outlined below.

Risk perceptions and biases

Tversky and Kahnemann have singled out three heuristics that influence judgement under uncertainty: Availability, representativeness, and anchoring/adjustment, all of which can have a biasing effect. Easily recalled events (availability) are judged more probable, i.e. more salient, familiar, recent, and imaginable events are perceived to be more likely. Exposure to an illness or death (e.g. in family or friends) may influence risk perception. This has empirically been confirmed in breast cancer for employees at an oncology center (Helzlsour et al., 1994) and women with a family history of breast cancer (Drossaert et al., 1996; Zakowski et al., 1997; Erblich et al., 2000a). Furthermore, individuals tend to place emphasis on

perceived similarities (representativeness). Thus, the extent to which a women feels that she resembles relatives, who have suffered from breast cancer, may influence her perception of risk. Individuals are also biased towards a preconceived idea about their level of risk when provided with a new risk information (anchoring and adjustment). The initial risk estimate is said to act as an anchor, which is adjusted after receiving new risk information. For this last heuristic, there is little evidence from the family history literature.

Illness representations and experience

In a theoretical model developed by Leventhal and co-workers (see Leventhal & Benyamini, 1997), which is based on the Self Regulatory Model, they distinguish five domains of illness representations are outlined. Illness representations refer to perceptions and believes about an illness including identity of the threat (its symptom and label), cause (e.g. infection, genetic, stress), time line (duration and development), consequences (including somatic and psychosocial), and controllability in terms of prevention and cure. These representations are likely to influence how individuals react to their own risk status.

The experiences of women with their relative's disease may have a profound effect on their illness representations. It is conceivable that women with the same "objective" familial risk have had considerably different experiences, i.e. a positive role model of a relative who survived breast cancer and coped well or a negative model with the relative suffering physically and mentally before dying. It seems likely that in such cases, women can acquire very different representations about the controllability of breast cancer. In one study conducted by Payne (1990), women's beliefs about cause and control of breast cancer were found to be associated with increased breast self-examination. Their prior experience with breast cancer in their family was, however, not assessed. To date, little empirical data is available on how awareness of familial risk and genetic predisposition may affect illness representations. It appears likely, though, that having different experiences might have an impact on representations of disease cause and possibly also on beliefs about controllability and cure.

Behavioral consequences

Women at increased breast cancer risk are confronted with a number of decisions to be made about risk management, i.e. what measures to take in order to respond to their increased risk for developing a potentially life-threatening disease. Possible actions to be taken include genetic testing, breast self-examination and other screening options, or even prophylactic surgery. However, the efficacy of any of these measures remain unclear and the women often lack clear advice on the best course of action (Rees et al., 2001). Several theoretical models

have been proposed to explain what human reasoning and decision-making is based on. Early theories assumed that decisions are based on an estimation of probability and utility in order to maximize positive outcome. Empirical research however has failed to provide clear evidence for this rational theory (Neumann & Polister, 1992). The subjective expected utility theory (SEU) now takes into account that subjective interpretation of both probabilities and utilities influences the decision. It seems plausible that experiences both personal and those of relatives strongly influence a person's interpretation of probability and especially the utility of a certain measure (e.g. efficacy of screening). However to date, only anecdotal evidence supports this theory (Rees et al., 2001, p. 1436).

The health belief model (HBM) was originally formulated in the mid-1960s (Rosenstock, 1966) to predict compliance with immunization and screening protocols. Here, four beliefs determine the readiness to engage in health behaviors: Perceived susceptibility, perceived seriousness of the health threat, perceived benefits of action, and perceived costs. This model has not directly been tested in women at familial breast cancer risk. However, Champion (1987) reported that perceived susceptibility predicted more frequent breast self-examination in the general population. It is also known that women with a family history show higher levels of perceived susceptibility and perceive breast cancer as more severe (Wellisch et al., 1991; Drossaert et al., 1996). However, the impact of family history on screening behavior is not fully understood yet (see below).

2.1.3. Psychological distress in women at familial breast cancer risk: Empirical evidence

According to cognitive stress theory (Lazarus & Folkman, 1984), the evaluation of a threat to one's life is a major factor leading to stress. Conceivably, women with a family history of breast cancer may be under continuous stress, which itself constitutes a threat to their health. A growing body of evidence suggests that women with at family risk of breast cancer (FR+) are likely to experience chronic psychological stress.

In an early study, Kash et al. (1992) examined 217 healthy women with a family history of breast cancer (mean age 44 years). Family history was defined as having 2 or more first-degree relatives (FDR) with breast cancer or 1 first-degree relative with premenopausal breast cancer or a mother and maternal grandmother with breast cancer. These women showed psychological distress (Brief Symptom Inventory) almost one standard deviation above the norm. Twenty-seven percent of the women exhibited levels above a standardized cut-off, which indicates a need for psychological counseling. The group further reported that increased cancer anxiety was negatively correlated with regular clinical examinations.

In a cross-sectional telephone interview study in 140 women with at least one first degree relative with breast cancer (average age 46 years), Lerman et al. (1993) found levels of intrusive thoughts which were comparable to clinical populations. However, depression scores were comparable to those observed in the general population. They also found negative associations of cancer-related intrusive thoughts and worries with mammography adherence.

One year later, Lerman et al. (1994) published data accumulated at three different cancer centers (n=179; n=238; n=363, respectively) from women aged younger than 50 with at least one first degree relative collected. While they confirmed findings of an association of psychological distress and mammography nonadherences, they could not find serious psychological morbidity. Levels of depression were reported to be within the normal range. However, results showed that one third of the women experienced breast cancer worries, which impair their daily functioning.

Baider and colleagues (2000) recruited healthy women at a conference on the subject of familial breast cancer. They report that women whose mother and sister had breast cancer (n=20) showed significantly higher intrusive thoughts and avoidance on the than women with either sister (n=34) or mother (n=176) with breast cancer. Overall, 53% of the women had scores of 63 or higher on the General Severity Index of the BSI, interpreted as psychological distress in the range of psychopathology.

Results from a large sample of 430 healthy women at high risk for breast cancer (Wellisch & Lindberg, 2001) suggest that a higher number of relatives with cancer, more symptoms of anxiety and more self-perceived vulnerability to breast cancer predict incidence of depression as measured by a self-report questionnaire.

In another publication from the same sample, Lindberg & Wellisch (2001) also report that strong general anxiety was common. However, general and screening-specific anxiety was not related to compliance with most of the screening procedures. However, there was a strong inverse association with the frequency of breast self-exams.

According to a study by Neise et al. (2001), 67% of 129 women with a family history of breast cancer showed medium or intense psychological strain. Nine out of 10 women described negative effects as occasional occurrences, impairing their mental and physical well-being.

None of the studies mentioned above included comparison groups of women without first-degree relatives with breast cancer. So far, only a few studies have investigated the distress levels in women at familial risk compared to a sample of women at normal risk.

In an early investigation, Wellisch et al. (1991) examined psychological distress in daughters of breast cancer patients (n=60) and 60 matched comparison subjects without a maternal history of breast cancer. They reported no differences in psychological symptoms, coping styles, breast self-examination practices, mammography practices, health knowledge, or body-image ratings between the groups.

Bovbjerg and Valdimarsdottir (1993) investigated psychological distress and natural killer cell activity in a sample of 43 women (mean age 39 years), eleven of which reported one or more first degree relatives with cancer. They could not find significant differences in life events over the past six months or total distress over the days before the assessment. However, women with a family history had lower levels of natural killer cell cytotoxicity.

A study by Valdimarsdottir et al. (1995) examined effects of undergoing mammography on breast cancer related psychological distress (52 women, mean age 40 years). Using established guideline for family history assessment (Garber et al., 1991), they found significantly higher levels of acute distress, non-specific distress (GSI of the BSI), avoidance and intrusive thoughts about breast cancer in the high risk groups (n=26) in comparison to normal risk women not undergoing mammography. While levels of acute distress were reduced to control group scores and no longer significantly different after notification of normal mammography results, GSI scores remained significantly elevated in women at higher familial risk.

Zakowski and co-workers (1997) determined predictors of intrusive thoughts and avoidance in a sample of 46 women at high risk versus 43 women at normal risk of breast cancer. They confirmed higher frequencies of intrusive thoughts and avoidance in high risk women both the day before and 4 to 8 weeks after their yearly mammogram. The group also reports that FR+ women whose mother had died from breast cancer experienced significantly increased intrusive thoughts.

In a sample of 93 women with a family history of breast cancer and 142 women with no such history, Gilbar (1998) investigated general distress (as measured with the Brief Symptom Inventory) and coping strategies. Significantly increased levels of general distress (GSI) were only found in women with familial risk who attended a regular check-up because of a symptom.

Confirming findings from Zakowski et al. (1997), Erblich and colleagues (2000a) reported elevated levels of breast-cancer related distress (measured by IES) in women at increased risk of breast cancer (FR+) whose mother had died of breast cancer in a study enrolling 57 FR+ and 91 FR- women. They also observed that women, who had cared for their mothers with

breast cancer, showed increased levels of cancer-related distress. Women who experienced caregiving as well as death had the highest levels of cancer-related distress and depressive symptoms.

In summary, women with a family history of breast cancer may experience a number of psychological problems, including increased anxiety and intrusive thoughts, which interfere to some extent with their daily lives. This was mainly reported in studies enrolling large numbers of women. We have limited understanding of why some women at familial risk of breast cancer show high levels of distress while others do not. According to the above outlined model by Rees et al. (2001), it would be expected that for instance women who have suffered bereavement, or whose relatives are currently undergoing treatment may show higher levels of distress. Two studies have provided empirical support for this assumption (Zakowski et al., 1997; Erblich et al., 2000a).

2.1.4. Biobehavioral model of familial breast cancer risk

Based on the empirical evidence outlined above, Bovbjerg and Valdimarsdottir (2001) recently developed a biobehavioral model to illustrate the interrelation of psychological distress, health behaviors, physiological variables and hereditary breast cancer risk. By integrating findings from formerly independent lines of research, this model provides the basis to derive testable hypotheses which link family histories of breast cancer and increased cancer incidence. A slightly modified version of the model is depicted in figure 1.

According to the literature (see above), cancer susceptibility genes like BRCA1 and BRCA2 (and potentially other unidentified genes) account for roughly half of the hereditary breast cancers (pathway **A-F**). On the other hand, only 16% of a sample of 263 women with breast cancer (and familial risk) were found to carry a BRCA1 mutation (Couch et al., 1997). Mutation carriers of BRCA1 and BRCA2 face a 35%-85% life-time risk of developing breast cancer (see Euhus et al., 2002). As has been shown above, considerable evidence supports the notion that family history of breast cancer often correlate with increased psychological distress as well as depressive symptoms (pathway **A-B**). Little evidence is available about which dimensions (e.g. general anxiety vs. disease specific worries) are affected most and whether subgroups of women at increased familial risk are more likely to exhibit increased levels of psychological distress. Rees et al. (2001) have hypothesized that those women with very recent and negative experiences with cancer in their family may experiences higher levels of stress (see above).

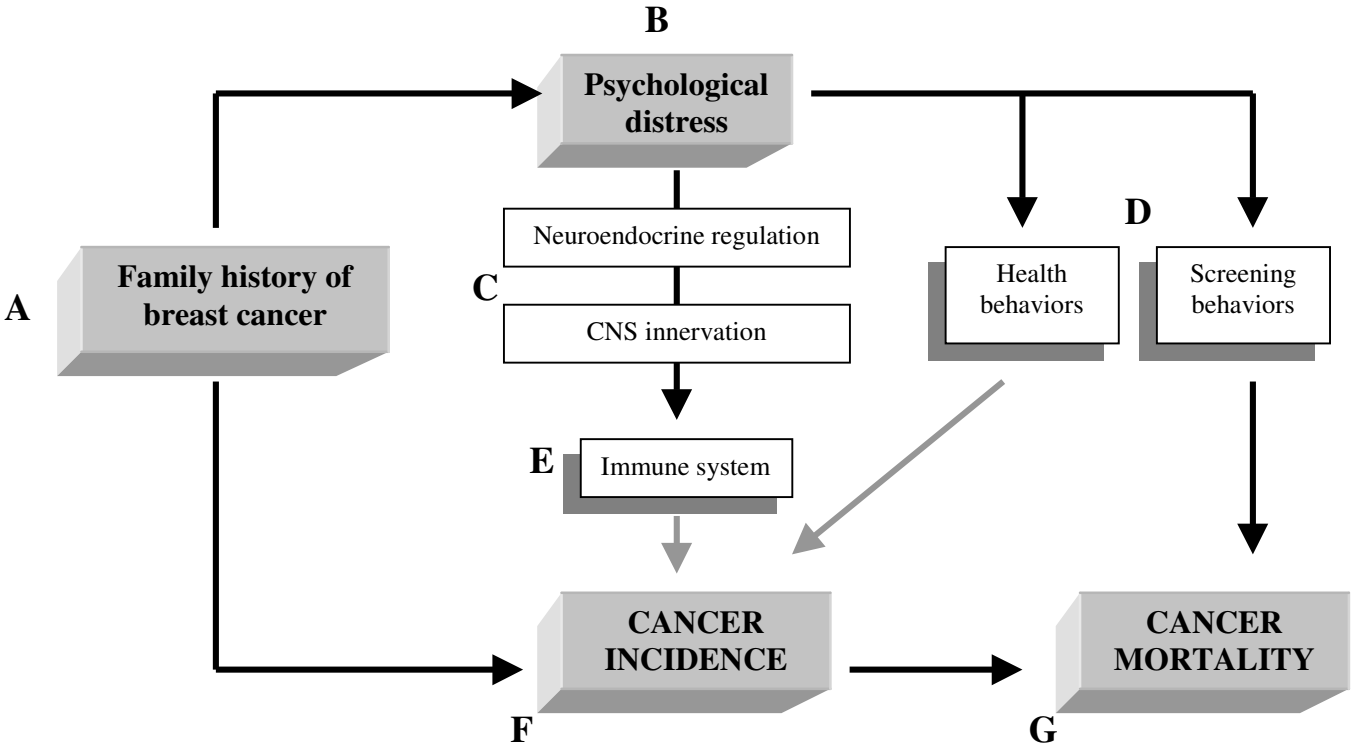

Figure 1: Schematic biobehavioral model of the interaction between psychological distress, familial risk of cancer, and cancer incidence (slightly modified after Bovbjerg & Valdimarsdottir, 2001). Illustrated are several potential pathways by which psychological distress may affect health outcome. Description in the text.

Compliance with recommended breast cancer screening remains the single most important behavioral variable with respect to reducing the risk of dying from the disease. When tumors are detected at an early stage, successful treatment is more likely. Recent studies suggest that early detection is associated with a reduction of mortality by up to 40% (pathway **D-G**; Helzlsouer, 1993; Chamberlain & Palli, 1993). It appears that among normal-risk women, higher levels of distress are associated with increased frequency of mammograms (meta-analysis by McCaul et al., 1996). There is, however, considerable evidence that distress can represent a barrier to breast cancer screening in women with a family history of breast cancer (pathway **B-D**). In this group, increased levels of anxiety have been found to be related to lower adherence to cancer screenings like mammography (Lerman et al., 1993) or less frequent breast self-exams (Kash et al., 1992; Lindberg & Wellisch, 2001, see above). The association between emotional distress and health behavior in women at increased breast

cancer risk may also depend on the conceptualization or measurement of distress. For, research has shown that cancer-specific distress is more associated with health behavior than general distress (Lerman et al., 1997). Lerman and Schwarz (1993) conclude that women who have high scores on measures of general anxiety are more likely to be seriously psychologically disturbed, and therefore more likely to avoid screening. However, the moderate worries assessed by measures of breast-cancer specific anxiety may be sufficiently high to motivate screening behavior but not so high that they cause women to avoid screening. In a recent study by Brain et al. (1999) enrolling 826 women with familial breast cancer risk, this was confirmed for the association of higher cancer-specific anxiety and more frequent breast self-exam (BSE), while higher levels of general anxiety were linked to excessive (inappropriate) BSE frequency.

Bovbjerg and Valdimarsdottir (2001) have suggested that there may be an inverse "U"-shaped association between psychological distress and compliance with cancer screening and self-exams. According to Decruyenaere et al. (2000), the inconsistent findings may reflect a facilitating or interfering interaction between problem- and emotion-focused action. Furthermore, several studies indicate that over- as well as underperformance may be predicted by high levels of distress (Epstein et al., 1997; Erblich et al., 2000b).

Empirical support that psychological distress affects immune measures via endocrine regulation and CNS innervation (pathway **B-C-E**) is abundant. A whole area of research called "psychoneuroimmunology" has investigated this interaction since the early 1970s. To review this literature is beyond the scope of the present report. Comprehensive overviews of the different research areas in the field can be found in Ader et al. (2001). A recent meta-analysis on the effects of depression and naturally occurring acute stressors on immune function has been published by Zorilla et al. (2001). It is important to note that there is a large body of evidence supporting that this immunomodulation in the periphery is transmitted from the central nervous system through secretion of stress hormones (pathway **C-E**), most prominently from the hypothalamo-pituitary-adrenal (HPA) axis, or via the sympathetic nervous system. Recent reviews of the anatomical and physiological mechanisms of immune-neuro-endocrine interactions were published by Sapolsky et al. (2000), Elenkov et al. (2000), and Besedovsky & Del Rey (1996). These interrelations are of great importance for the study presented here, since these endocrine mediators are the focus of the research questions.

The involvement of the immune system in tumor control (pathway **D-F**) is increasingly recognized (see Carbone et al., 2002). Natural killer cell activity (NKCA) is thought to play a crucial role in cancer surveillance especially at early stages of the disease (Whiteside &

Herberman, 1995) and low NKCA has recently been found to predict cancer incidence in a large prospective study (Imai et al., 2000).

Only very few studies have tested parts of the model empirically. Some of the first reports published have addressed the connection between family history, psychological stress, and immune function. Indeed, data has become available that family history of breast cancer may be linked to physiological changes. Decreased basal levels of NK activity (Bovbjerg & Valdimarsdottir, 1993) and increased reactivity both of NK cell numbers and activity to a laboratory stressor have been reported (Valdimarsdottir et al., 2002). This was found in relatively small samples. In the former study, a trend was seen towards increased levels of stress in women with familial risk. Statistically controlling for psychological distress reduced the association of family history and NK activity but did not eliminate it, suggesting that several psychological as well as biological or other factors are involved.

One very recent investigation has included measures of basal endocrine activity, thus providing information about another part of the model (pathway **A-B-C-E**). Cohen et al. (2002a) studied a sample of 80 daughters (mean age 32 years) of breast cancer patients. They reported increased levels of emotional distress and cortisol as well as catecholamine concentrations. Furthermore, NKCA and in vitro cytokine production (IL-2, IL-12, IFN-gamma) was decreased compared to age- and education-matched controls (n=47, mean age 31 years). They also found moderate but significant inverse correlations of psychological stress measures and stress hormones with NKCA and cytokine production.

As described above, multi-factorial disease models may be useful in breast cancer risk research. However, as Dalton et al. (2002) have recently concluded that methodological weaknesses of many studies, which investigate the association between stress and cancer incidence have made it very difficult to find empirical support for the validity of such models. The authors highlight that the question of underlying biological mechanisms, which link stressful events and cancer, remains unresolved.

According to the modified model by Bovbjerg and Valdimarsdottir (2001) briefly described above, a key element of the interaction between psychological distress and cancer in high risk population are the neuroendocrine systems, most notably the hypothalamo-pituitary-adrenal axis and the sympathetic nervous pathway. After briefly reviewing the most influential stress theories in the next section, these two major stress response systems of the body are discussed with respect to their anatomy and physiology. The kinetics of the acute stress response is presented with particular emphasis on the conceptual and ecological importance of reactivity

and recovery. Finally, experimental paradigms used in human research are described and their potential for eliciting endocrine responses are summarized. This will be followed by the fourth section discussing the biological relevance and long-term effects of alterations in these responses as postulated by Bruce McEwen in his theory of allostatic load (1998).

2.2. Theory of stress: Anatomical, physiological and psychological fundamentals

2.1.1. Stress concepts: a historical overview

Although hundreds of scientific research papers, essays, chapters, and books have been published on the subject of stress in the past decades, no generally accepted scientific definition of stress exists. Concepts have changed considerably from a rather physiological and stressor-unspecific definition such as Selye's in the 1930s to more dynamic and multidimensional approaches in McEwen's theory of allostatic load. Pacak and Palkovitis (2001) have outlined the changes that the concept underwent throughout the decades. The most influential of theses theories are briefly described below.

Homeostasis

Walter Cannon was the first to introduce the term "homeostasis" as defined by "coordinated physiological processes which maintain the steady states in the organism". The sympathetic nervous system was a key factor in his theory. He considered it the main homeostatic system that allowed the body to respond to disturbances of the homeostasis and promote the survival of the organism (Cannon, 1929).

Selye's General Adaptation Syndrome

In the mid 1930s, Hans Selye reported typical physiological alterations which he observed after exposure to a variety of stressors in rats. Selye called these alterations the "pathological triad": adrenal enlargement, gastrointestinal ulceration, and thymicolymphatic involution. Based on this concept, he developed his theory of stress (actually coining the term stress in a biomedical sense), which he defined as the nonspecific response of the body to any demanding or threatening situation. These situations are called "stressors", the actual cause of stress. In his theory, such demands on the body included bacterial infection, toxins, x-irradiation, and various stimuli such as surgery and muscular exercise. While Selye himself did not deny that stressor-specific components of a response may exist, he confined his concept of stress to the shared nonspecific component. Selye mainly focused on the HPA axis

as the primary mediator of the stress response. Indeed, he was able to reproduce the three components of the pathological triad by administering ACTH (see Selye, 1976 for a comprehensive review).

Selye also introduced the term of a "general adaptation syndrome" with its three successive phases alarm, resistance, and exhaustion. He later stated that most of the stressful stimuli induce two types of responses. First, a general stress response, which is common to all stressors and involves the release of ACTH and adrenal corticosterone. Secondly, an individual stress response mediated by "conditioning factors".

Further refinements: Psychological approaches

Criticisms of Selye's theories have focused on the fact that this notion of nonspecificity seems to rule out psychological mechanisms in determining the response to a stressor. Baum et al. (1997) however have argued that this is not necessarily the case if we "assume that the non-specific nature of stress is limited to our initial response. If only the initial phase of responding to stress is non-specific, late reactions may be mediated by a variety of factors, including appraisal" (p. 67).

Mason (1971) pointed out that not all stressors induced a HPA activation so that the presence of the pathological triad may not indicate the occurrence of stress. He also suggested that the elicitation of emotions like anxiety and fear are the basis for the similar endocrine responses to stress.

Lazarus and Folkman have proposed a psychological concept of stress, where stress is defined as "a particular relationship between the person and the environment that is appraised as taxing or exceeding his or her resources and endangering his or her well-being" (Lazarus & Folkman, 1984). Lazarus suggested that unless we perceive a situation as threatening, we will not experience stress. Already in the 1950s, empirical evidence was available supporting the notion that being unconscious during a life-threatening situation prevented the occurrence of physiological and psychological stress responses (Symington et al., 1955; Adler, 1943). In this theory, cognitive appraisal, coping, and anticipated consequences play a crucial role. A given stimulus or situation can be appraised as irrelevant (i.e. the event will not affect me), benign (the event is positive), and harmful or threatening. Stressful appraisal may involve evaluation of harm or potential loss, threat of danger, and challenge. After a situation is judged to be threatening or stressful (primary appraisal), secondary appraisals are made to consider our response options, i.e. judge the dangers or benefits of different modes of coping with perceived threats.

Ursin, Levine and colleagues more recently proposed the Cognitive Activation Theory of Stress (CATS, Levine & Ursin, 1991; Ursin et al., 1988). Here they try an integration of available physiological and homeostatic stress concepts. According to their definition, stress responses occur whenever there is a discrepancy between what the organism expects (set value) and what really exists (actual value of the same variable). Thus, the stress response functions as an alarm indicating an imbalance in the homeostasis (Levine & Ursin, 1991). Since the response is uncomfortable, it drives the organism to provide specific solutions to abolish the source of alarm, as well as the alarm itself. The stress response thus serves to mobilize all physiological resources to improve performance. The phases of this response need to be understood as an alarm occurring within a complex cognitive system with feed-back and feed-forward control loops. One important implication of this approach is that the stress response is dynamic and develops over time (Eriksen et al., 1999).

2.2.2. Anatomical and physiological basics of the stress response

As shown above, early theories of stress have put a strong emphasis on the endocrine system. It should be noted that a wide array of hormones are secreted by the body in response to acute stress. As briefly described in section 2.2.3., these include corticotropin-releasing hormone (CRH), adenocorticotropic hormone (ACTH), prolactin, growth hormone, arginine vasopressine (AVP), epinephrine and norepinephrine, as well as glucocorticoids. All of these hormones play an important role in preparing the body for the response to a stressful challenge. In addition, other proteins such as neuropetides are secreted in response to acute stress (see Stout et al., 1995). Reviewing the specific effects of each protein is, however, beyond the scope of this report. As outlined above, the sympathetic nervous system and the hypothalamo-pituitary-adrenal (HPA) axis are integral parts of the major stress theories. The respective products of these two systems, catecholamines and glucocorticoids, have received special recognition in endocrine stress research. Cannon for instance considered the sympathetic nervous system the key factor in the body's effort to maintain homeostasis and respond to external or internal challenges. Hans Selye developed his "general adaptation syndrome" based on the observation that very different stimuli elicited a physiological response with one common feature, an activation of the hypothalamo-pituitary-adrenal (HPA) axis. Although psychological factors such as appraisal have received increasing recognition in more recent stress theories, HPA and SNS activation remain key variables in these concepts. In the sections below, an emphasis will thus be put on HPA and sympathetic mechanisms.

First, the anatomical and physiological fundamentals of these two stress systems will be briefly reviewed.

2.2.2.1. The Hypothalamo-Pituitary-Adrenal (HPA) Axis: Anatomy & physiology

The hypothalamus, a small brain area situated below the third ventricle, releases hormones into the portal venous system, which stimulate pituitary function. The secretory function of the hypothalamus is regulated by a neural network of higher brain structures, which transmits external or internal signals. For example, threatening stimuli perceived by the eye induce the secretion of stress hypothalamic hormones such as corticotropin-releasing hormone (CRH) into the portal venous system. The pituitary is a small structure located in the sella turcica at the base of the skull (in front of the optic chiasma above the pituitary fossa). The pituitary amplifies the action of the hypothalamus. The adenocorticotropic hormone (ACTH) is one of the at least nine different hormones released into the blood stream from the pituitary after CRH stimulation. The target tissue of this peptide is the adrenal cortex, which in turn releases glucocorticoids. While among the GCs, cortisol is the most important in humans, corticosterone is the primary GC in rodents. Each secretory episode of cortisol is preceded by a pulse of ACTH from the pituitary. The secretion of ACTH and cortisol follows a circadian pattern characterized by increasing basal levels towards the beginning of the waking period (i.e. in the morning in humans). The HPA axis also responds to stress with release of ACTH and cortisol.

Glucocorticoids released from the adrenal cortex directly inhibit the activity of the HPA axis at the level of the pituitary, hypothalamus, and other higher centers. Two different types of receptors are mediating these effects. The high-affinity type I receptors (mineralocorticoid receptors, MR) with limited distribution but high expression in the hippocampus, are thought to be responsible for controlling basal levels at the circadian nadir. In contrast, the low-affinity type II receptors (glucocorticoid receptors, GR), which show a more widespread expression in central nervous system, seem to control peak and stress levels of glucocorticoids.

2.2.2.2. The Sympathetic Nervous System: Anatomy & physiology

The autonomic nervous system (ANS) regulates the function of all innervated tissues and organs throughout the body except for the skeletal muscle fibers, i.e. it connects with the involuntary muscles in organs such as the lungs, the stomach, and kidneys. It forms the major efferent component of the peripheral nervous system. Since its activities cannot be controlled consciously, the ANS has been considered to be “independent”. It can be divided into three

parts: the sympathetic (adrenergic) nervous system (SNS), the parasympathetic (cholinergic) system, and the enteric nervous system. While the former two originate within the central nervous system, the latter lies within the gastrointestinal tract. Of theses three, the SNS is not only the most extensive but also the most diverse in terms of physiologic function (see overview in Elenkov et al., 2000).

The SNS originates in nuclei within the brain stem and gives rise to preganglionic efferent fibers. These leave the CNS through the thoracic and lumbar spinal nerves (thoracolumbar system). The majority of the sympathetic preganglionic fibers terminate in ganglia located in the paravertebral chains. These lie on either side of the spinal cord, while the remaining fibers terminate in the prevertebral ganglia (in front of the vertebrae). From these ganglia, postganglionic sympathetic fibers run to the innervated tissues and organs. Postganglionic sympathetic fibers mainly release norepinephrine. However, the chromaffine cells of the adrenal medulla are innervated by typical preganglionic sympathetic fibers and release epinephrine and – to a lesser extent – norepinephrine (ratio approximately 4:1, see Elenkov et al., 2000). Norepinephrine and epinephrine are the principal end products of the sympathetic nervous system.

2.2.3. Kinetics and effects of the response to acute stress

Modern stress theories have significantly broadened the definition of stress response by integrating a multi-dimensional perspective (i.e. responses of endocrine, cardiovascular, and psychological variables). Furthermore, they have added a dimension of time, defining the acute stress response as a cascade of interdependent reactions in different systems of the body. Levine, Ursin, and co-workers have proposed a conceptualization of stress responses as a dynamic construct, which starts with brain activation within milli-seconds and finally terminates through a neuroendocrine activation, which could take 10-15 minutes or even longer to reach the peak concentrations.

Ursin and Levine (Levine & Ursin, 1991) and later extensions of the theory (Eriksen et al., 1999) have postulated that the initial stage of the stress response is characterized by positive feedback and feed-forward mechanisms. Here, many of the responses facilitate or reinforce further development of the stress state. Ursin and Levine highlight that feedback from prior experience may facilitate the response. In contrast, at later stages of the response, homeostatic mechanisms become activated, which help the system to return to resting levels. This is primarily achieved by the slower responding systems such as glucocorticoids, which are thought to dampen the total stress response. The “tail of the acute response” has thus been

regarded as a homeostatic device to reestablish physiological balance (Munck et al., 1984). However, more recent approaches have recognized that glucocorticoid secretion may not only play a role in terminating the response (i.e. suppression) but also exert permissive, stimulatory and preparatory actions. These antagonistic effects are thought to be mediated at different concentrations via the GR and MR receptors (see Sapolsky et al., 2000).

Brain activation

According to the CATS (cognitive activation theory of stress, see above), the triggering event for a stress response is that the brain records a sudden discrepancy between set value and actual value and sets off an alarm. Wakefulness and changes in EEG patterns are the primary responses to this discrepancy. This process may require only milliseconds to occur and is accompanied by metabolic and circulatory changes, which set the stage for the next series of events. These develop within seconds and can be monitored by functional brain-imaging techniques.

Behavioral activation

Behavioral changes will initially be characterized by the orienting response. Later on, an attention or targeting response directed at the stimulus or the source of the discrepancy between expectancies and reality (see above) can be observed. These initial, rather unspecific (or stereotyped) responses will then gradually develop into goal-directed behavior. The behavioral response depends on the situation as well as previous experiences.

Early neuroendocrine activation

Changes in psychophysiological systems such as the cardiovascular system are induced by activation of the autonomic nervous system. These responses are mediated by hormones like CRH and catecholamines and take considerably longer than the earlier behavioral responses triggered by motor neurons. Hormone secretion still occurs within seconds of the stressor onset. This first wave of endocrine activation (Sapolsky et al., 2000) involves enhanced secretion of catecholamines and hypothalamic release of CRH into the portal circulation. Approximately 10 seconds later, ACTH is secreted by the pituitary. Shortly thereafter, pituitary release of gonadotropins and prolactin can be observed. Most stressor also induce a moderate arginine vasopressine (AVP) response by the pituitary. All these proteins are released within the first minute after stress onset.

Late neuroendocrine activation

The main endocrine events are the secretion of the pituitary (ACTH) hormones and the secondary hormones such as cortisol, insulin, and thyroxin. The time axis of these responses

ranges from seconds for the pituitary release and minutes for secondary hormones. Glucocorticoids may require 10-15 minutes to reach their peak levels.

2.2.4. Reactivity vs. Recovery. Definition, conceptual and ecological importance

Physiological and psychological responses to an acute stressor can be described in terms of reactivity (i.e. the stress-induced increase or decrease) as well as recovery (i.e. the return to baseline after the stressor has terminated). Since the terms reactivity and recovery are central to this study both with respect to the conceptual framework as well as the research design. Therefore, brief definitions may be useful. According to Linden and co-workers (1997), reactivity testing in the lab typically comprises "an initial rest, or baseline period, followed by a period during which the subject is exposed to a stressor [...]. Simultaneous physiological measures document the ensuing change which is then called *reactivity*. *Recovery*, in its simplest form can be defined as a poststress rest period that provides information about the degree to which the elevation (i.e., reactivity) in the physiological and psychological parameters being measured persists after the stressor has ended." In this study, the term "response" is used to describe the overall pattern, i.e. reactivity *and* recovery.

Linden et al. (1997) highlight that although Selye's theory already hypothesized that it is the activation beyond the resistance stage which contributes to disease, assessment of stress recovery has been widely neglected in empirical research. They conducted a literature review of 4 major scientific journals for the years 1994 and 1995. It was reported that out of 105 studies investigating the physiological stress responses, 69 (63%) clearly stated that a recovery phase was part of the protocol; but only 24 articles (23%) actually report recovery data. This seems unfortunate on conceptual as well as methodological grounds. As will be discussed below, endocrine recovery plays an important role in recent theorizing on allostatic load and the implications of altered stress responses for health consequences. Furthermore, there is some evidence from the animal and human stress literature, which reveals that positive findings were obtained in some instances in the recovery period. These would not have been apparent in reactivity comparisons only (see Linden et al., 1997). Moreover, recent animal models suggest that recovery may be more sensitive to stressor intensity and background stress than reactivity (e.g., Garcia et al., 2000; Marquez et al., 2002).

2.2.5. Neuroendocrine response to experimental stress in humans

A number of different experimental stressors have been developed for human research. They are employed to measure the physiological and psychological stress response and to draw

inferences about how humans react to stressful stimuli encountered in their all-day lives. Depending on the theoretical background of interest, these vary with respect to their characteristics. Commonly used stress paradigms range from passive stressors (e.g. cold pressure test, heat stress) to cognitive challenges (mental arithmetic or the STROOP test), and more social stressors (public speaking). A large number of studies have been conducted in the past decades to investigate the endocrine response to these stressors. They strive to determine the kinetics of such responses. Other studies have focussed on characteristics which account for stress response differences between individuals. Mental arithmetic and public speaking are among the most commonly used experimental stressors in this research. As will be shown in the next section, these paradigm can strongly activate the SNS and/or elicit a stress response of the HPA axis.

Mental arithmetic

Early studies focused on comparing mental arithmetic to stressors of a passive nature. One of the first studies (Le Blanc et al., 1979) used a 2-minute arithmetic test in 12 healthy subjects. It was discovered that norepinephrine levels showed similar responses after the cognitive task and the cold pressure test. Epinephrine levels were induced significantly stronger by mental arithmetic.

A 4-minute series of continuous subtraction (Ward et al., 1983) also induced significant elevations in both plasma epinephrine and norepinephrine in eight male healthy subjects (mean age 40 years) Endocrine responses were compared to blood pressure measurement, which was considered nonstressful. Again, the epinephrine response was greater than stress-induced changes by a painful passive stressor (cold pressure test and venipuncture) and physical stressors (knee bends and handgrip). Dimsdale (1984) showed slight elevations of epinephrine and highly significant increases (compared to a 20 minute resting period) in norepinephrine after 3 minutes of serial subtraction in 11 healthy male subjects (average age 30 years).

Barnes et al (1982) examined the effects of a 12 minute mental stress protocol (5-minutes digit span test and 7 minutes of serial subtraction) in a sample of 10 healthy young and 10 elderly men (mean age 26 vs. 68 years). Responses were greater for both epinephrine and norepinephrine compared to levels during a control procedure. In a study with a large sample size including 45 men and 45 women (mean age 33 years for both groups), significant elevations were seen in epinephrine after a 5-minute serial subtraction task with distracting background noise. Norepinephrine levels were not affected by this task. Also, no differential

effects of sex on catecholamine response were reported (Davidson et al., 1984). No change in norepinephrine but significant increases in epinephrine were found in a study enrolling 20 healthy young male volunteers (mean age 30 years) after a 15-minute period of serial subtraction and the digit span test (Del Rio et al., 1994). The studies mentioned above did not investigate changes in HPA axis hormones (ACTH, cortisol).

Jorgensen and colleagues (1990) investigated endocrine responses to 15 minutes of mental arithmetic in a sample of 14 healthy subjects (5 men, 9 women, mean age 36 years). In this study, hormone recovery patterns after stressor termination were also investigated. Epinephrine levels increased significantly during the stressor but rapidly returned to basal levels after termination of the task. In contrast, norepinephrine and cortisol did not increase significantly during the 15 minutes of stress. At 15 minutes post stressor termination, cortisol was however significantly elevated over baseline levels.

Williams et al. (1991) showed that in a sample of 28 healthy men (mean age 41 years), norepinephrine and epinephrine increased significantly after a serial subtraction task (18 minutes). No changes were seen in cortisol. The results were compared to the responses of the same subjects to a word identification task (counterbalanced order).

Sgoutas-Emch and coworkers (1994) used a 12-minute mental arithmetic stressor (two 6-minute serial subtraction problems) with random 100 dB noise blasts interjected at approximately 17 second intervals during the second part. Their sample of 22 healthy male undergraduate students (age range 18-31 years) showed significant elevations in epinephrine and norepinephrine. However, only a subsample (n=11) identified as high heart rate responders in a previous session exhibited significant increases in cortisol.

A sample of women with different obesity phenotypes (all had a body mass index greater 30) and six normal weight women (mean age 27 years) were subjected to a mixed cognitive stressor. The task comprised completion of two puzzles and mental arithmetic (20 minutes) (Pasquali et al., 1996). Blood samples were collected before and during (intervals) the stress test and before and after a control condition (saline). No significant increases were seen in either ACTH nor cortisol. Catecholamines were not measured. Another small study (Modell et al., 1990) reported only a slight, non-significant increase in plasma cortisol in 5 healthy women (age range 22-30 years) after a battery of standardized mental tasks (arithmetic problems, 15 minutes, with frequent reminders of remaining time).

In summary, most studies provide evidence for increases in catecholamine levels after brief sessions of mental arithmetic tasks. Given that catecholamines belong to the mediators of the first wave of the stress response (see Sapolsky et al., 2000), time of sampling as well as

duration of the stressor might be a crucial issue, There is no consistent support for a robust activation of the HPA axis by this type of experimental stress as indicated by plasma cortisol and ACTH levels.

Public speaking plus mental arithmetic

Mental arithmetic tests represent a purely mental task with little social involvement apart from the interaction with the experimenter. Public speaking, on the other hand, can be considered as a complex source of stress with an interpersonal component as well as anticipation of possible failure and a cognitive task. This paradigm has been found to induce anxiety and vigilance (Dimsdale, 1984). It seems that mental arithmetic only causes such elevations in self-report stress when a harassment condition is added (Earle et al., 1999).

One early study (Kemmer et al., 1986) examined stress responses in patients with diabetes and a healthy control group (healthy control group n=9, age range 20-32). Here, mental arithmetic (45 minutes) was compared to a public speaking task. For the latter, subjects were asked to speak about their life history and future plans in front of a video camera that supposedly transmitted their speech to an audience of psychologists. Both stressors significantly induced increases in epinephrine and norepinephrine. Only the speech task produced a cortisol response. A more recent report by Al'Absi et al. (1997) explored mental arithmetic and speech task in a sample of 52 healthy men (mean age 27 years). Participants were subjected to 24 minutes of mental arithmetic (3 cycles à 8 minutes) and 24 minutes of speech tasks (also 3 cycles), separated by a 30 minutes recovery period and a 10 minute break. The two stressors were presented in a counterbalanced order. Both stressors produced substantial increases in ACTH and cortisol. Nevertheless, the speech task produced greater elevations in both parameters directly post stress. This difference was still apparent after 30 minutes of recovery. Catecholamines were not assessed.

Cacioppo et al. (1995) used six consecutive serial subtraction problems and a 6-minute speech task. Subjects were asked to imagine themselves defending against a false accusation of shop-lifting (22 elderly women, mean age 67 years). This protocol produced significant increases in plasma epinephrine, norepinephrine, and ACTH. No changes were seen in cortisol.

The group around Dirk Hellhammer and Clemens Kirschbaum in Trier has established a standardized laboratory stressor. It consists of a resting period (30 minutes after insertion of catheter), an anticipation phase (10 minutes preparation), a public speaking task in front of an audience (5 minutes), and a serial subtraction task (5 minutes) (Kirschbaum et al., 1993). During the first part, the participants take over the part of a job applicant and are asked to

introduce themselves to a group of three “staff managers” who respond in a standardized way. Subjects are allowed 10 minutes for preparation and 5 minutes for delivery. Subsequently, they are asked by the “managers” to perform a 5-minute mental arithmetic in front of this audience. The whole session is video- and audiotaped.

This so-called Trier Social Stress Test (TSST) has been used in a number of studies both from this groups as well as other researchers. Kirschbaum and coworkers have published a number of reports establishing the response of salivary cortisol to this stress protocol. The assessment of salivary cortisol enables them to obtain more frequent samples in an non-invasive way. This approach facilitates the investigation of response kinetics and recovery patterns. Cortisol in saliva has been found to be highly correlated with plasma levels (see Kirschbaum & Hellhammer, 1994).

The TSST reliably induces cortisol responses. A study enrolling monozygotic and dizygotic twin pairs (13 and 11, respectively) showed significant cortisol increases after the TSST. The response peaked around 30 minutes after stressor onset (Kirschbaum et al., 1992a). Kirschbaum et al. (1992b) also published an analysis of pooled data from three independent studies carried out with the TSST with a total sample of 135 healthy subjects (64 men, 71 women). This analysis confirmed the effectiveness of the stressor to produce a significant cortisol response and peak levels at 30 minutes post stress onset. The test has been used in a number of other studies examining sex differences and effects of oral contraceptives (Kirschbaum et al., 1999), age (Kudielka et al., 1999; 2000), polymorphisms of glucocorticoid receptors (Wüst et al., 2002) and recently also included measures of glucocorticoid sensitivity of immune function (Rohleder et al., 2001; 2002; 2003).

Gerra et al. (2001) have elegantly shown that a compound stressor comprising the STROOP test (10 minutes), mental arithmetic (10 minutes), and a speech task in front of an audience (10 minutes) enhances epinephrine, norepinephrine, ACTH, and cortisol plasma concentrations in 20 healthy male subjects (mean age 26 years). They could further show that when the protocol was repeated 8 days later with the same subjects, ACTH and cortisol responses were smaller while no change in catecholamine reactivity was observed.

Biondi & Picardi (1999) conclude that stress protocols, which consist of both mental arithmetic and public speaking, seem to induce endocrine responses characterized by sympathetic, adrenomedullary and adrenocortical activation. Since only few studies allow for separate analysis of arithmetic and public speaking, it is unclear whether the two types of stress differentially affect the sympathetic pathway and the HPA axis. Still, Picardi & Biondi highlight, that the catecholamine response to the combined protocol and mental arithmetic

only, is very similar. They argue that because the combined task was also shown to activate the HPA axis, the speech task is likely to be responsible for triggering the cortisol response.
A recent meta-analysis (Dickerson & Kemeney, 2002) including 165 stress studies showed, that overall, laboratory-based psychological stressors significantly increase cortisol levels. However, a wide variability was observed. For instance, the time of day the study was conducted and intervals of cortisol assessment was associated with differences in cortisol reactivity. However, when statistically controlling for these methodological aspects, uncontrollability and social-evaluative threat significantly predicted cortisol response. This effect was strongest for paradigms containing both qualities (like the TSST). Contrarily, stressors without either component failed to produce cortisol increases.

2.3. Chronic stress and acute endocrine stress responses

2.3.1. Animal studies

There is a long tradition in the animal literature to explore the effects of prior exposure to a chronic stressor on acute stress responses. On methodological grounds, these studies can be distinguished whether they used "homotypic" or "heterotyic" stress paradigms. Homotypic refers to using the same stressor for chronic exposure and for eliciting an acute response. For instance, animals are subjected to repeated daily restraint stress for several days and then are re-exposed to restraint to examine acute response. In a heterotypic paradigm, two different stressors are used. Here, one would expose the animals to repeated daily restraint for several days. Subsequently, foot-shocks can be used to elicit an acute responses. Typically, in case of homotypic stressors, a habituation over time is seen in the endocrine response, i.e. the stressor elicits weaker and weaker responses (see McCarthy et al., 1988 for an early review). Still, heterotypic stress designs have been found to lead to a sensitization of the acute response. Repeated exposure to restraint stress for several days increases the endocrine response to a novel stressor such as forced swim test. Such facilitating effects have been reported in particular for the catecholamine response (see McCarthy et al., 1988). Below, more recent findings are summarized from studies investigating the effects of prior exposure to stress on HPA and sympathetic responses to a novel (heterotypic) stressor.
Konorska and colleagues (1989) exposed rats to different combinations of heterotypic chronic and acute stressor combinations. Animals were exposed daily to 30 minutes of either restraint, footshock or cold swim for 26 days. Their response to a novel stressor (restraint, footshock or cold swim but not the stressor they had been exposed to chronically) was then compared to

control rats. While basal catecholamine levels were not affected, chronically stressed animals showed significantly greater norepinephrine and epinephrine responses to the novel stressor.

Bhatnagar et al (1995) chronically exposed rats for 21 days to an intermittent cold stressor (4C for 4 h a day). No changes in basal corticosterone or catecholamines were found. However, upon exposure to a novel stressor (20 minutes of restraint), plasma levels of corticosterone were significantly elevated compared to animals not exposed to chronic stress. However, stress-induced levels of epinephrine and norepinephrine were not significantly different.

In another study using the same paradigm, Bhatnagar & Dallman (1998) confirmed increased reactivity to heterotypic (restraint) stress after chronic exposure to intermittent cold for corticosterone and ACTH. They further found evidence for facilitation of the HPA axis responses by chronic stress. This was indicated by increased *fos*-expression in the posterior paraventricular thalamus, parts of the amygdala, and parvocellular paraventricular hypothalamus. Since the presence of Fos protein reflects neural activity, this suggests that these brain areas may be the neuroanatomical correlates for a facilitating effect of prior stress exposure.

Increased c-fos expression in the amygdala after acute social defeat was also reported in rats which had previously been subjected to 1 hour of restraint stress for 10 days (Chung et al., 2000). No differential effects of chronic stress were observed on acute corticosterone response. One has to bear in mind that glucocorticoid levels were only determined 60 minutes after stress termination. Thus, the authors might have missed the peak of glucocorticoid secretion. McCormick et al. (1998) found that rats, which had undergone a mild handling stressor, showed strong corticosterone responses to one hour of maternal separation. Their response was significantly stronger in comparison to non-handled controls.

A series of studies has shown that even prenatal exposure to stress may enhance acute stress reactivity. For example, in a recent study conducted by Weinstock et al. (1998), pregnant rats were subjected to unpredictable and intermittent noise and light stress. When male offspring of stressed and unstressed rats reached the age of 4.5-5 months, they received three footshocks in a experimental session. Here, norepinephrine reactivity was significantly increased in prenatally stressed rats.

There is evidence that even a single session of prior stress may be sufficient to increase the HPA response to a novel stressor. In a very recent study by Johnson et al. (2002), rats were exposed to inescapable tailshocks and later on subjected to an immunological (LPS injection) and a psychological stressor (pedestal exposure). In contrast to animals not previously

subjected to the tailshock stressor, corticosterone and ACTH response to both novel stressors were significantly elevated in previously stressed rats.

In summary, prior exposure to stress may facilitate endocrine responses to a novel acute stressor. This effect was observed in the HPA axis as well as the sympathetic pathway. It appears that duration of the prior stress exposure is not particularly relevant for this facilitation. Antonio Armario and his group have hypothesized that intensity of the chronic stressor may be more important. From a series of experiments, they conclude that prior exposure to severe stressors leads to desensitization of the HPA axis. On the other hand, repeated prior cycles of moderate stress facilitate HPA activation by a novel acute stressor, resulting in an enhanced response (Andres et al., 1999).

2.3.2. Human studies

Gump and Matthews (1999) have recently reviewed 17 cardiovascular studies that reported the extent of acute stress reactivity as a function of background stressors in the participants' lives. These stressors were often measured by checklists of life events that occurred within the last 6 to 12 months, so one did not know whether the stressors had already resolved by the time of testing. Nonetheless, the review suggested that a slight majority of studies showed enhanced acute-stress cardiovascular reactivity as well as delayed recovery in participants who reported high background stress. A post-hoc analysis of the studies revealed that ongoing, important background stressors were associated with enhanced responses to acute stress. Background stressors, which were resolved, infrequent, or avoidable were linked to less pronounced acute stress responses. In a study by Lepore et al. (1997), individuals who had high levels of chronic stress for at least 9 months had greater heart rate and blood pressure responses to acute stress. Compared to individuals who had chronic stress of intermediate length or past episodic stressors, the high level group exhibited a significantly delayed post-stress recovery.

The authors speculate in another paper (Gump & Matthews, 1998) that repeatedly coping with an ongoing stressor leads to sustained vigilance for possible threat. This may prime the individual to respond strongly to acute stressors. On the other hand, stressors that are resolved, infrequent, or avoidable do not lead to sustained vigilance.

The effect of chronic stress exposure on acute endocrine stress response has rarely been investigated in humans. Benschop et al. (1994), studied job stress among high school teachers. Subjects (n=27, mean age 40 years) were recruited for extremely high or extremely low scores on a daily hassle scale. Participants were then subjected to a 30 minute laboratory stressor

(three-dimensional puzzle under time pressure and presentation of their solution to another person). They found no differences in the autonomic or endocrine reactivity to acute stress in participants reporting high or low job stress.

A field study by Ockenfels et al. (1995) investigated diurnal cortisol patterns and cortisol reactivity to naturally occurring acute stressors. Participants were beeped on a pre-programmed wrist-watch randomly 6 times a day. An acute stressor was defined as a significant events since the last beep, dealing with a problem the past 5 minutes, anticipating any stressful events in the next hour. The sample comprised 60 employed (17 males, 43 females, mean age 33 years) and 60 unemployed subjects (18 males, 43 females, mean age 39 years). There was only one measure of salivary cortisol available for analysis, which was obtained approximately 25 minutes after the event. The unemployed group exhibited significantly higher levels of self-reported chronic stress. No group difference was found in cortisol reactivity. However, due to the lack of a baseline sample, it is difficult to interpret this "acute reactivity" measure.

Van Eck et al. (1996) compared salivary cortisol responses to a speech task in 42 "high stress" (Perceived Stress Scale PSS$\geq$16) and 45 "low stress" (PSS$\leq$10) male white collar workers (mean age 42 years). Subjects were specifically recruited for their PSS scores. Participants presented to the laboratory between 11 a.m. and 1 p.m., and were subjected to a speech task. The task consisted of 10 minutes of preparation, 5 minutes of presentation, and a 15 minute recovery period. No significant differences were found in the cortisol response between high stress and low stress subjects. Furthermore, no associations of stress-induced cortisol concentrations or basal levels over a period of 5 days were found with anxiety, anger, depression, psychosomatic symptoms, coping style or personality variables. Basal levels and acute response (area under the curve) were only moderately correlated.

Pike et al. (1997) tested psychological, immune, and endocrine responses to a laboratory stressor (12 minutes of mental arithmetic with standardized prompts) in 23 male volunteers. Participants were categorized into control subjects (n=11, mean age 36 years) and a chronic life stress group (n=12, mean age 40 years). This categorization was based on a multistep evaluation of life event frequency, severity, and threat. They reported differential effects of chronic life stress on self-reported distress, NK cell numbers and plasma epinephrine induced by the task with stronger reactivity in the chronic stress group (Pike et al., 1997). However, no differences were seen during the recovery period (30 minutes post stressor termination).

Roy et al. (1998) investigated cortisol and cardiovascular reactivity and recovery in 90 young probationary firefighter (mean age 25 years). The stressor consisted of a cognitive challenge

and a speech task where participants were being interviewed as a performance review, which was conducted by a senior fire officer. They found no differences in subgroups with low or high life events within the past year (median split) in relation to their reactivity or recovery in either cortisol or cardiovascular measures. Still, there was an association of higher social support with greater cardiovascular reactivity and faster recovery and a significant interaction between life events and social support for cardiovascular measures.

In a study enrolling female spousal caregivers of Alzheimer's patients, Cacioppo et al. (2000) examined cardiovascular and neuroendocrine reactivity (epinephrine, norepinephrine, ACTH and cortisol) to a combined mental arithmetic (6 minutes) and speech task (3 minutes preparation, 3 minutes delivery). Here, the women were asked to imagine that they are being harassed by an obnoxious bill collector. Cardiovascular measures and blood samples were collected directly pre and post the stressor. Consistent with previous research from this group, caregivers (n=27) had higher levels of self-reported distress compared to age-matched non-caregivers (mean age of total sample 67 years). They reported significantly elevated scores of perceived stress (PSS), depressive symptoms (Hamilton depression rating scale) and negative affect (Positive and Negative Affect Schedule PANAS). Caregivers also showed increased cortisol reactivity, however cardiovascular parameters, as well as catecholamine responses did not differ from controls.

Matthews et al. (2001) investigated cardiovascular and neuroendocrine stress reactivity and recovery in a sample of 62 volunteers (31 men, 31 women, mean age 35 years) with high or low background stress. To obtain a composite measure of chronic stress, they averaged scores on the Perceived Stress Scale (PSS), the Job Environment Inventory (JEI) and the Dyadic Adjustment Scale (DAS) administered twice within an average of 35 days. The stress task consisted of a 5 minute mental arithmetic and 5 minutes of public speaking (video-taped). Participants were asked to defend themselves against a false accusation of shop lifting. Linear regression revealed that high stress subjects showed lower systolic blood pressure reactivity and faster recovery after the stressor. No significant cortisol response to the stressor was observed. Also, there was no statistically significant difference in norepinephrine or epinephrine reactivity and recovery.

One study examined responses to acute stressors in individuals with subclinical scores on the BDI (Light et al., 1998). The authors report increased cardiovascular response and a slight increase in catecholamine reactivity in a subgroup of women with high BDI scores (top 25%) compared to women with low scores (lower 25%). While group differences were statistically significant, the effects were modest.

As described above, there is some evidence from the literature that family history of breast cancer can be a potent chronic stressor. Only one study has so far explored the effects of having a family history of breast cancer on acute physiological stress reactivity. Valdimarsdottir et al. (2002) reported that in a group of 16 women at familial risk, acute reactivity of self-reported distress, heart rate, NK cell numbers and NKCA to a speech task and mental arithmetic compared to a group of women at normal risk (n=32).

In summary, animal models have quite compellingly demonstrated that prior exposure to chronic stress can facilitate acute endocrine responses to a novel stressor. Two animal studies furthermore suggest that stress reactivity is more sensitive to the effect of chronic stress than basal levels (Konorska et al., 1989; Bhatnagar et al., 1995). There seems to be an association with stress intensity of the chronic stressor. Human research in this area has been scant. However, some studies suggest that a facilitating effect of prior exposure to chronic stress like caregiving can enhance acute physiological response to a laboratory stressor. Only few studies, which have used self-report measures of perceived stress or life events to identify subjects with high background stress, have succeeded in finding differential endocrine responses to acute stress.

2.4. Biological relevance of altered stress response dynamics

The above sections have reviewed the theoretical, anatomical, physiological and psychological fundamentals of the acute stress responses. In section 2.2.5., acute stressors used in human research have been reviewed and the endocrine responses normally seen in healthy subjects were described. It was further shown from the animal literature as well as from first evidence in humans that prior exposure to chronic stress can facilitate future responses to a novel stressor. As we will see in the next section, there is only limited evidence for the biological relevance of such stress response alterations. However, new data has recently begun to emerged and Bruce McEwen has proposed a conceptual framework to explain and test why such alterations in acute stress responses may be related to increased health risks. This theory will be outlined in the next section.

2.4.1. The concept of "allostasis" and "allostatic load"

New theories have broadened the definition of stress. Presently, not every physiological response to environmental and psychosocial situations is simply regarded as "stress". Sterling and Eyer, coming from cardiovascular research, have introduced the term "allostasis" (which

translates to “maintaining stability through change”) to describe the readjustment of a steeping for resting and active states of the body (Sterling & Eyers, 1988). This change in set-point is the key feature which distinguishes allostasis from homeostasis. Roughly 10 years later, Bruce McEwen extended the concept by defining “allostasis” as the process for actively maintaining homeostasis as well as stability through change. He also coined the term “allostatic load” referring to the cost, or “wear and tear” on the body taxed by repeated cycles of allostasis (McEwen, 1998; McEwen & Seeman, 1999). That is, each of these adaptive processes has a potential cost to the body when allostasis is either called upon too often or is inefficiently managed. Drawing references to a number of older concepts like Cannon and Selye (see above), this theory states, that while the acute stress response is highly adaptive, repeated cycles of eliciting this response may have detrimental effects.

The concept of allostasis and allostatic load circumscribes a cascade of cause and effect. It starts with the release of the primary stress mediators, i.e. the hormones of the HPA axis and the sympathetic nervous system. McEwen and colleagues have outlined a number of primary effects, and secondary and tertiary outcomes that ensue from this initial catecholamine and glucocorticoid release.

The behavioral and neuroendocrine responses to an internal or external stimulus are coordinated by the brain as the integrative center. This coordination, however, depends on individual differences in genes, development, and experience. They constitute the coping abilities of the individual. The system, being challenged by a “stressor”, has to prove its ability to adapt. It has to achieve a new point of stability by changing the setpoint for a number of physiological systems. According to McEwen (2000), “allostatic load” refers to the price the body pays for being forced to adapt to adverse psychological or physical situations. It describes the wear and tear on the body that ultimately may lead to an increased susceptibility for disease.

Allostasis and allostatic load are general concepts that apply to all stress response systems of the body. It is therefore important to understand the mechanisms that play a role in each of these systems. The primary stress mediators have different effects in different target tissues. Whenever hormones are secreted, both the short-term and long-term consequences have to be considered.

For each system of the body, there are short-term adaptive actions (allostasis), which are protective. Long-term effects of the same actions, however, can be damaging (allostatic load). Adjusting one’s heart rate to sleep, awakening or exercise by catecholamine secretion for

example promotes adaptation. Contrarily, repeated surges of blood pressure may enhance arteriosclerosis. In primates, repeated elevations of blood pressure over periods of weeks and months accelerate arteriosclerosis and may thereby increase the risk of myocardial infarction (Kaplan et al., 1991). In the brain, acute elevations of stress mediators such as glucocorticoids and catecholamines have been found to promote retention of memories of emotionally charged events. Chronic overactivity of the HPA axis, however, has been linked to neurodegeneration of GC-sensitive brain areas such as the hippocampus and seems to be associated with cognitive impairment in animals and humans (see Lupien & McEwen, 1997 for review). In the immune system, glucocorticoids act in synergy with catecholamines to promote "trafficking" of the immune cells to organs and tissues where the response is needed. They also regulate the release of chemokines and cytokines. Glucocorticoids are generally thought to downregulate expression of proinflammatory cytokines such as IL-1, IL-6, and TNF-alpha, thereby limiting an inflammatory response (see Haddad et al., 2002 for review). If the neuroendocrine regulatory loop of immune responses is interrupted, autoimmune disease may ensue (see Webster et al., 2002, for review). On the other hand, immunosuppression and increased susceptibility to disease may occur if these mediators are secreted chronically or not shut-off properly (see McEwen et al., 1997).

2.4.2. Subtypes of allostatic load

As shown in figure 2, allostatic load may be divided into four subtypes, all of which are associated with increased health risks. The first one can simply be described as "too much stress", i.e. high *frequency* of exposures to novel stressors. This subtype is most closely related to what is generally called "chronic stress". In fact, it describes animal models of repeated stress exposure often used to mimic chronic stress in humans. The three other forms of allostatic load in McEwen's theory describe *alterations* in acute stress responses.

Type II refers to the failure to habituate or adapt to the same stressor. A full-blown stress response is needed the first time a stressor is encountered. However, repeated exposure should lead to a decrease in reactivity in order to avoid detrimental effects of chronically high levels of stress hormones.

The third type involves the failure to adequately shut-off a mounted stress response after the stressor is no longer present. Again, a fast initiation of responses in the main stress systems is needed to provide the body with energy and other resources. This high level of arousal is physiologically costly. It must therefore be shut-off as soon as the threat is gone in order to avoid long-term health risks.

Finally, an inadequate (hyporeactive) stress response (type IV) may be harmful to the host by allowing other systems, such as the inflammatory cytokines, to become overactive.

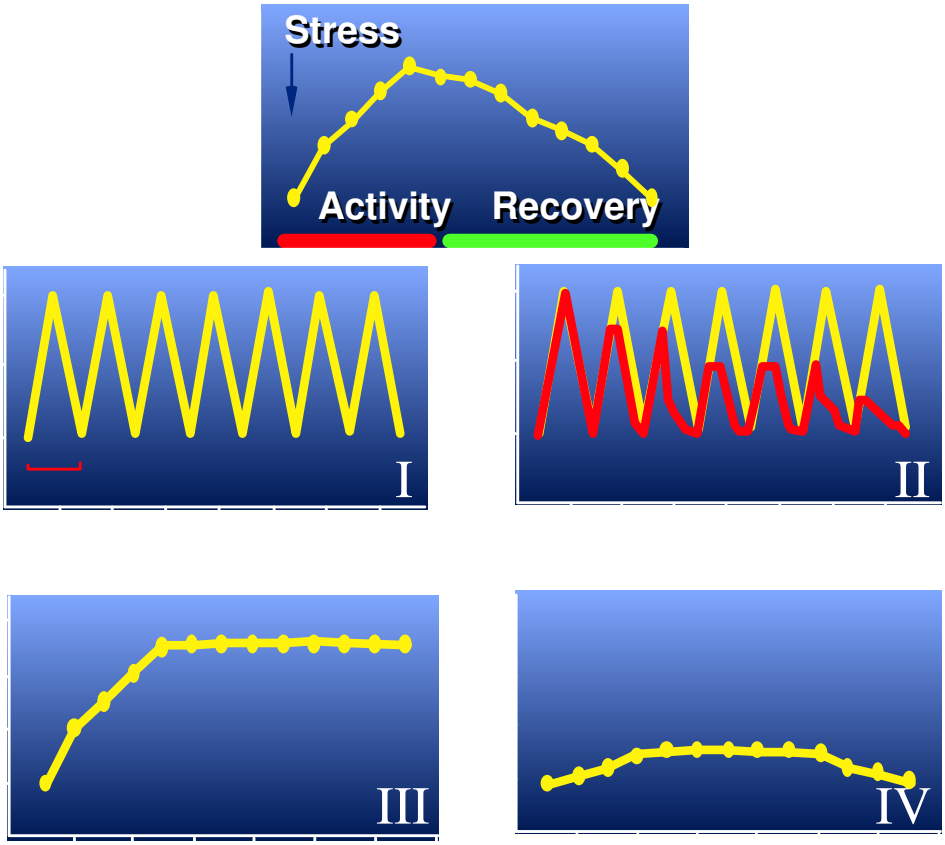

Figure 2: Subtypes of "allostatic load". Explanation in the text (taken from McEwen, 1998).

2.4.3. Mediators, effects, and outcomes

McEwen further refined his theory in a later paper (McEwen & Seeman, 1999), now categorizing the components with respect to their position in the cascade. Here, he differentiates primary mediators, primary effects, secondary and tertiary outcomes.

Primary mediators

This term refers to the chemical messengers that are released as part of allostasis. The main primary mediators described by McEwen are glucocorticoids, catecholamines (i.e. epinephrine and norepinephrine), and DHEA. The latter is a functional antagonist of cortisol, which is considered to have deleterious effects when chronically low.

Primary effects

Primary effects describe a broad array of cellular events in the target tissue, which result from the release of the primary mediators. These include enzymes, receptors, ion channels, or structural proteins, which are induced genomically or are being phosphorylated via second-messenger systems. Several of the effects caused by different mediators converge at the level of gene transcription. This is for example seen with glucocorticoid and cAMP pathways (see Yamada et al., 1999). It is thus conceivable that the outcomes described below are the result of more than one primary mediator. The measurement of primary effects has rarely been included in studies examining the interrelation of mediators and outcomes.

Secondary outcomes

McEwen and colleagues summarized the cumulative outcomes of the primary effects in a tissue/organ specific manner. The so-called "secondary outcomes" reflect the sum of changes induced in a certain physiologic system such as the cardiovascular system. They are a result of the primary effects (cellular changes), which were in turn caused by the primary mediators. For example, the secretion of cortisol and catecholamines (primary mediators) causes changes in blood glucose levels, which in orchestration with other primary effects increase blood pressure. Increased blood-pressure would be the secondary outcome of this cascade.

However, only a limited number of such secondary outcomes have been formulated. Most of them are related to the cardiovascular system. Even fewer have been empirically validated. Furthermore, there is still a lack of definitions referring to other systems such as the immune system or the brain. It has been proposed that brain-related parameters should include measures of declarative and spatial memory. Furthermore, assessments of related changes in brain morphology, especially in the hippocampus, are recommended (McEwen, 2001). With regard to the immune system, such measures may include delayed-type hypersensitivity and immunization challenges (vaccination). The frequency and severity of common cold symptoms may also be a useful tool. First evidence was recently provided by Cohen et al. (2002b). In a prospective study, the authors reported that healthy subjects who exhibited stronger cortisol responses to a laboratory speech task had a greater incidence of upper respiratory tract infections in the 12 weeks following the experimental session. However, this was only true for those participants with high cortisol reactivity who also reported a high number of negative life events.

Tertiary outcomes

Tertiary outcomes as defined by the concept of allostatic load are actual disorders or diseases. These are a result of allostatic load and can be predicted by high levels of secondary outcomes

and primary mediators. Myocardial infarct would represent such a tertiary outcome with high blood pressure as a related secondary outcome. Also, while cognitive decline would count as a secondary outcome, Alzheimer's disease would be the resulting tertiary outcome. Other diseases, which can be investigated from this perspective may include cancer and autoimmune disease.

This new classification can be a tool to help relating progression of pathophysiology from primary mediators to secondary outcomes and tertiary outcomes, i.e. diseases. It may also be helpful for identifying clusters of secondary outcomes, which are relevant to a given disease. The theory is useful for understanding the potential long-term consequences of alterations in the responsivity of primary mediators (cortisol, catecholamines) to acute challenges. This could be investigated cross-sectionally, e.g. testing endocrine stress responses to an experimental task such as an experimental stressor. Longitudinal studies may provide information about the predictive value of alterations in the responses of primary mediators. McEwen and colleagues provide a model of pathways, which mediate such consequences. The model can be used to operationalize these pathways so they can be studied in cross-sectional and longitudinal investigations. First data to support the clinical relevance of the theory have recently begun to emerge, particularly with respect to cognitive function and psychiatric disorders (McEwen, 2000). For example, Lupien et al. (1998) found that persistent increases of basal cortisol levels were predictive of reduced hippocampus volume and deficits in several memory tasks over a five-year period.

Both for conceptual (Linden et al., 1997; McEwen, 1998) as well as empirical reasons (e.g., Garcia et al., 2000; Marquez et al., 2002), it appears to be promising to include recovery measures of endocrine stress response since this period may be more sensitive to background stress as well as more important in terms of long-term health implications.

2.5. Conclusion and research questions

In summary, research has demonstrated that being at increased familial risk for developing breast cancer is perceived as a chronic stressor. Statistically significant increases of distress levels were reported in studies enrolling large numbers of women. Based on the modified schematic model by Bovbjerg and Valdimarsdottir (2001), the neuroendocrine stress systems play an important role because they mediate the hypothesized effects of distress on the immune system.

The main objective of this study was to investigate whether women at familial breast cancer risk exhibit altered endocrine stress responses. It is further tested whether this may be

explained by increased psychological distress. Using the terminology of the modified biobehavioral model presented in section 2.1.4., the pathway A-B-C was tested. The recent theory of allostatic load provides an argument for possible pathways by which alterations in acute stress response may contribute to adverse health consequences. It further suggests that measures of acute stress response may be helpful tools as a marker for "allostatic load". This cross-sectional study examines for the first time the stress response patterns of primary mediators (cortisol, catecholamines) in a population at high risk of tertiary outcomes (cancer).

Both animal and human studies suggest that exposure to chronic stress affects the endocrine stress response systems. This has been shown for the HPA axis as well as the SNS. The effects of chronic stress on acute stress responses have been investigated in animals. A relatively small number of human studies has also been publish in this area. Findings from the animal literature suggest that chronic stress leads to a facilitation of endocrine stress responses both in HPA as well as SNS parameters. Findings from human studies are less consistent. Some of the human studies conducted have found similar effects as in the animal models, others did not report altered acute stress responses in subjects under chronic stress. It appears, however, that a facilitation of HPA and SNS stress responses can be detected in subjects under important and ongoing chronic stress. The mixed findings are likely to be due to methodological differences including but not limited to the type of chronic stressor used, the experimental stress paradigm, and the study population.

In the study presented here, we used being at increased risk for breast cancer as a chronic stress paradigm. The project was designed to investigate the effects of this "chronic stressor" on perceived stress and depressive symptoms in a sample of healthy women with or without increased risk for breast cancer. We further tested acute stress reactivity and recovery of cortisol and catecholamine secretion to a standardized laboratory stressor (speech task and mental arithmetic).

3. METHODS

3.1. Study sample

3.1.1. Subjects

Ninety-six women, recruited in the New York metropolitan area from two cancer surveillance hospitals and advertisements in local newspapers, successfully underwent screening for eligibility for the study (inclusion and exclusion criteria see below). From this sample of 96

women participating in the study, 83 women had complete data in requisite cardiovascular, endocrine, and self-report measures and were thus included in the analyses.

3.1.2. Inclusion and exclusion criteria

Participants were eligible if they were between 25 and 50 years of age, premenopausal, had not taken birth control pills in the past two months, were not currently pregnant and at least six months after their last pregnancy, did not report a personal history of chronic disease or neoplasm, were not currently taking any drug known to influence immune or endocrine function and did not have relatives currently in active cancer treatment. Participants were excluded if they reported personal histories of any neurological or psychiatric disease, including substance abuse, depression and anxiety disorders, as well as any endocrine disease like diabetes or thyroid diseases that might affect endocrine responses.

3.2. Classification of increased familial risk of breast cancer

Based on their self-reported family histories of breast cancer, participants were classified as being at increased risk of developing breast cancer (FR+, n=17) or normal risk (FR-, n=66). The classification was based on the statistical model of Claus et al. (1994, see below) using a cut-off score of 11% lifetime risk. The women completed the Family History Questionnaire (FHQ), a questionnaire asking detailed information about the cancer incidence in their family with special reference to breast and ovarian cancer. The information obtained with the questionnaire is then used to calculate familial risk of developing breast cancer according to a statistical model that takes into account the number of affected first- and second degree relatives and their age. The procedure to calculate the individual risk according to Claus is outlined below.

To help estimate the risk of a woman with particular constellations of affected relatives, Claus and co-workers computed tables from the data set of a large scale case-control study conducted by the Center for Disease Control in the United States. Previous analyses had revealed that a woman's risk of breast cancer was strongly related not only to the presence of a positive family history of breast cancer but, more specifically, to the number and type of relatives affected with breast cancer as well as the ages at which those relatives became affected (Claus et al., 1990).

The data set obtained for the Cancer and Steroid Hormone Study, a multicenter, population-based, case-control study, consisted of 4730 patients with histologically confirmed breast cancer (age range 20-54) and 4688 controls. The patients were registered between December

1, 1980, and December 31, 1982 at eight Surveillance, Epidemiology, and End Results Centers of the National Cancer Institute. Control subjects were frequency-matched to patients according to the geographic regions and 5-year categories of age. Patients and control subjects with a previous history of breast cancer or a breast biopsy with unknown outcome were excluded from the study. Further details about the inclusion and exclusion criteria of this study can be found in Wingo et al. (1990).

Goodness-of-fit tests were used to compare the observed age-specific risk patterns with those predicted under the best fitting genetic model. The results of these analyses provided evidence for the existence of a rare autosomal dominant allele leading to increased susceptibility to breast cancer (Claus et al., 1991). The strength of this approach is that it is model-based and derived from an extremely large data set. In contrast to another widely used risk estimation model by Gail et al. (1989), it does not take into account non-family related risk factors such as age at first child-birth and number of previous breast biopsies. The aim of the Claus model was to address the issue of risk calculation solely for that subset of women who are at potentially high risk of breast cancer, that is, women with a family history of breast cancer. For these women it is thought that "the number of relative(s) affected with breast cancer as well as the ages at onset of any affected relatives may be the most important risk factors, more so than factors such as age at first birth and age at menopause" (Claus et al., 1994, p.648).

In this paper, the authors provide seven tables that give an estimate of an woman's cumulative risk based on the number and type of relative affected, taking into account the age at onset of the affected relative(s). Probabilities are computed based on the current age of the woman. Tables give predicted cumulative probabilities for women with one first-degree relative, one second-degree relative, two first-degree relatives, mother and maternal aunt with breast cancer, mother and paternal aunt, one maternal and one paternal second-degree relative, or two second-degree relatives (both maternal or paternal) affected with breast cancer.

3.3. Procedure

3.3.1. Time table

Potential participants were screened in a telephone interview and an introductory session was scheduled to sign consent forms and allow the women to adapt to the setting in which the experiment would take place. The study consisted of two sessions scheduled one week apart.

Table 1 shows measures and sampling schedules for the study both for the questionnaires as well as physiological data.

Table 1: Flow chart of assessments conducted for the study in order to obtain background variables, control variables, self-report data and family history information.

Variables	Telephone interview	Visit 1	Home	Visit 2
Inclusion/exclusion criteria	●			
Background variables				
Ethnicity		●		
Marital status		●		
Living arrangement		●		
Education		●		
Employment		●		
Income		●		
Control variables				
Age	●			
Height, weight, BMI		●		
Sleep, caffeine, nicotine, alcohol				●
Menstrual cycle phase				●
Self-report variables				
Visual Analog Scales				●
Beck Depression Inventory (BDI)			●	
Perceived Stress Scale (PSS)			●	
Brief Symptom Inventory (BSI)			●	
Family History Questionnaire		●		
Physiological data				
Cardiovascular				●
Endocrine				●

Visit 1

All participants came to the laboratory for a brief initial visit (20 minutes) to provide informed consent and to fill in a series of questionnaires. During this session, a small blood sample was obtained and any questions the women might have had were answered. The blood sample was collected for the purpose of another study and the results are not reported here. This introductory session served to allow the women to habituate to the laboratory environment and meet the primary experimenter who would be present during the experimental session. This visit 1 was introduced into the protocol in order to minimize the level of baseline distress at the experimental session. It was further used to collect self-report data and hand out a packet of questionnaires that the participants were asked to fill in at home and bring back for the next session. Participants received financial compensation of US$20 at the end of this visit for their time and effort.

Visit 2

On the day of the stress test, subjects reported to the laboratory between 8:00 AM and 10:00 AM. This session was rescheduled when the participants reported any symptoms indicative of a cold or flu in the past three days. They were asked to refrain from drinking more than two alcoholic drinks the night before and more than one cup of coffee in the morning of the session.

Furthermore, they were asked not to take any medication in the 24 hours prior to the stress test. Upon arrival in the laboratory, an IV catheter was placed in the non-dominant arm. The catheter was kept patent with saline drip during the experimental session. Subsequently, an ambulatory blood pressure monitor was hooked up and an inflatable cuff was put on the participant's dominant arm. The monitor was started and the subjects were allowed a habituation period of 20 minutes to reduce the effect of the IV line and cuff placement on the outcome variables. Blood samples were drawn at baseline, immediately after stressor termination (15 minutes post baseline), as well as 30 and 45 minutes after baseline for hormone measurement. Figure 4 shows the sampling schedule for cardiovascular and endocrine assessments.

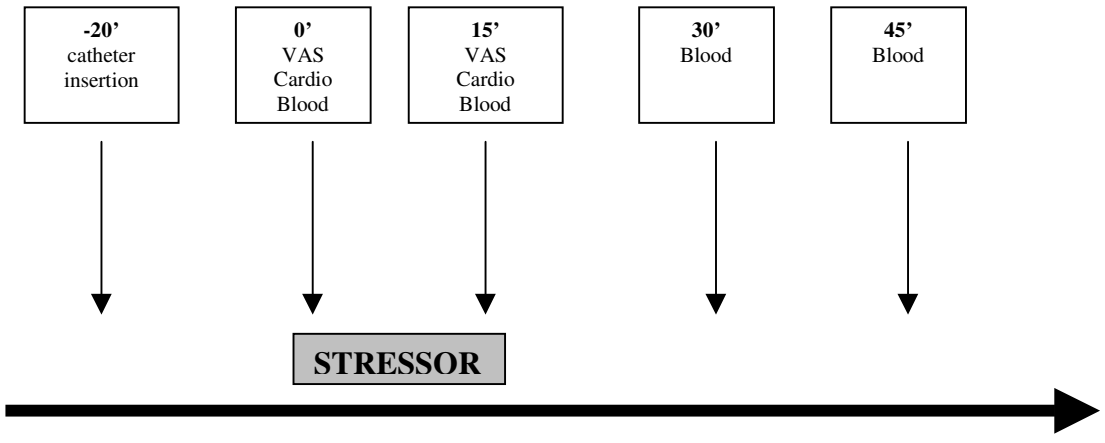

Figure 3: Time course of assessments during the experimental session

3.3.2. Randomized group assignment

The FR- group was randomly assigned to either the stress (n=36) or control condition (n=30) while all FR+ subjects underwent the acute psychological stressor (see below). This was necessary because of the relatively small number of subjects with Claus scores above 11 that could be recruited for this study. The unequal group sizes in the FR- sample are due to

missing data after randomization in the cardiovascular, endocrine, or self-report measures. For the analyses, all subjects were included in order to increase statistical power.

3.3.3. Experimental stressor

Speech Task and Mental Arithmetic

The stress test was a modified version of the Trier Social Stress Test (TSST, Kirschbaum et al., 1993), comprising a 5 min speech task and a 5 min mental arithmetic test with harassment. The test that has been introduced into psychobiological research by Kirschbaum and Hellhammer is one of the most widely used experimental paradigms in human stress research. Studies from the Trier Lab as well as other groups using it have consistently found reliable elevations in cortisol and catecholamines (see Biondi & Picardi, 1999, for review). As noted above, a recent meta-analysis (Dickerson & Kemeney, 2002) concluded that stressors like the TSST, which include a social component, are most likely to produce a stress response in the HPA axis.

For the speech task, participants were asked to imagine they had been caught for a traffic violation and had to defend themselves at the traffic court. They were allowed three minutes for preparation and two minutes for delivery. Speeches were delivered in front of a video camera, and the women were told that their performance would later be rated by experts for content and style. During the following mental arithmetic task, subjects had to add numbers out loud at the pace set by an audiotape (duration 5 minutes). This task requires speed as well as concentration from the participant. A series of one-digit numbers is presented to the subjects, who have to add the value of the new number to the previous one. This task is specifically demanding since the most salient stimulus is the solution of the addition rather than the new number. Throughout the task, the experimenter interjected standardized comments to the women participating in the study that they needed to work harder or faster.

Control Task

The sampling schedule and procedure for the non-stress control group (control FR-) was exactly the same as in the two groups undergoing the stress condition except for the nature of the task. The control condition was included in order to ensure that the stressor worked in our hands and produced a reliable stress response in self-report (VAS) as well as cardiovascular and neuroendocrine parameters. It consisted of two 5 min sessions of a non-stressful reading assignment, in which the women were told they could read the material provided by the experimenter at their leisure and would not be tested later on.

3.4. Background variables

The following background variables were obtained in order to compare group differences that might potentially affect endocrine stress response patterns and thus confound results. Variables assessed included ethnicity (White (non-Hispanic), African-American, Hispanic, Asian, other), marital status (never married, currently married, separated, divorced, widowed), living arrangement (live alone, live with roommate who is not partner, live with spouse or partner, live with parents, other), education (less than 7^{th} grade, Junior High School, partial High School, High School graduate, partial college or specialized training, standard college or university graduate, graduate professional training), employment, and income.

3.5. Control variables

Gender, age (Deuschle et al., 1997), weight (e.g. Epel et al., 2000), caffeine and nicotine use (e.g. Al Absi et al., 1998; Lovallo et al., 1996; Gilbert et al., 2000), meals (Kirschbaum & Hellhammer, 1994), posture at sampling (Hennig et al., 2000), sleep disturbances (Leproult et al., 1997), menstrual cycle phase (Kirschbaum et al., 1999), birth control pills and other estrogens taken (see Kirschbaum & Hellhammer, 1994), medications as antidepressants, asthma and other drugs (see Adam & Gunnar, 2001), may all well influence endocrine levels.

To control for variables known to have an effect on the endocrine system, we used self-report questionnaires to assess weight, height, and information on the menstrual status (days since last period). We also assessed sleep, nicotine, caffeine, and alcohol intake. As reported above, women were only included in the study when they were not taking birth control pills two months prior to the study.

Weight and height information was used to derive the body mass index. This measure is computed by dividing the weight [kg] by the square of height [m]. The BMI is a well-established marker of obesity and cut-off values have been developed in large samples of subjects. The World Health Organization has published a classification system of obesity, subdividing the BMI scores in a number of categories: BMI scores below 18.5 are considered to be underweight, scores between 18.5 and 24.9 are “normal”, while scores from 25 to 29.9 are considered “overweight”. Obesity is classified into three groups: Obesity class I (BMI 30.0-34.9), Obesity class II (BMI 35-39.9) and class III (BMI $\geq$40.0) (WHO, 1997).

Recent evidence has suggested that women with BMI scores above obesity cut-off levels show marked alterations in the endocrine system both with respect to basal concentrations in cortisol and catecholamines as well as endocrine responses to acute psychological stress (Epel et al., 2000; Vicennati & Pasquali, 2000, Pasquali & Vicinnati, 2000).

The different stages of the female menstrual cycle are known to be associated with changes in the hormonal system. Recently, Kirschbaum and colleagues (1999) found that menstrual cycle phase (follicular vs. luteal) and use of contraceptives had a profound effect on salivary cortisol response to the Trier Social Stress Test as well as to injection of ACTH. However, no such associations were found for blood cortisol levels. Following the suggestions of Kirschbaum and Hellhammer (1994), we assessed menstrual cycle phase (days since last period) and excluded women that had taken birth control pills within the past two months.
Intake of caffeine, nicotine and alcohol as well as hours of sleep were obtained for the three days prior to visit 2. Subjects were asked how many hours of sleep they had had in each of the three nights before the stress test. They also filled in how many alcoholic drinks they consumed the night before, 2 days ago and 3 days ago. Other items collected information about number of cigarettes and cups of coffee consumed the day of visit 2, the day before, 2 and 3 days earlier.

3.6. Psychological variables

3.6.1. Visual Analog Scales (VAS; see Ahearn, 1997 for review)

Participants' distress at baseline was assessed using a set of visual analog scales. They were asked to indicate the extent to which they experienced each of ten moods, including fatigue, anxiety, confusion, depression, energy, anger, tension, relaxation (scoring reversed), frustration, and nervousness by making marks across 100mm lines. The two ends of each line are marked by e.g. "not anxious at all" on the lower end and "as anxious as I could be" on the higher end (see appendix). Depending on how far away from the lower end the participant has marked the line, a score ranging from 0 to 100 is assigned.
Example:

Please put a slash through this line to indicate how anxious you feel right now

Not anxious at all _____ As anxious as I could be

Visual analog scales are widely used for the assessment of current mood because they are simple to complete, ensuring a high rate of compliance. The simplicity of assessment and speedy analysis of the VAS makes these scales especially suitable for repeated measurements within a short period of time.

At the baseline assessment, participants were asked to rate how they were feeling "right now" and immediately after the task period participants were asked to rate how they felt "during the tasks". To increase reliability, average scores across all 10 VASs were computed to form a mean distress scores for the baseline period. The VAS scales can be found in the appendix.

3.6.2. Beck Depression Inventory (BDI) (see Beck et al., 1988)

The 21-item BDI self-report questionnaire used in the study assesses depressive symptoms. The BDI was originally derived from clinical observations about the symptoms and attitudes displayed frequently by depressed psychiatric patients and infrequently by non-depressed psychiatric patients (Beck et al., 1961). These clinical observations were systematically consolidated into 21 items that cover symptoms and attitudes *in the past month,* specifically the presence and intensity of emotional, cognitive, and somatic aspects.

Each item can be rated from 0 to 3 in terms of intensity. The BDI is scored by summing the ratings given to each of the 21 items resulting in a possible score range from 0-63.

The items were generated with the intent to measure intensity of depression rather than being selected to reflect a certain theory of depression. The 21 symptoms and attitudes were (1) mood, (2) pessimism, (3) sense of failure, (4) lack of satisfaction, (5) guilt feeling, (6) sense of punishment, (7) self dislike, (8) self-accusation, (9) suicidal wishes, (10) crying, (11) irritability, (12) social withdrawal, (13) indecisiveness, (14) distortion of body image, (15) work inhibition, (16) sleep disturbance, (17) fatigability, (18) loss of appetite, (19) weight loss, (20) somatic preoccupation, and (21) loss of libido.

While the questionnaire was originally designed to be administered by a trained interviewer, it is most often self-administered. The BDI takes roughly 5-10 minutes to complete in the self-administered version.

Beck et al. (1988) have reviewed the psychometric properties of the BDI found in studies in the 25 years after its publication. Twenty-five studies had addressed internal consistency in psychiatric and non-psychiatric populations. In the 15 non-psychiatric studies, the BDI had a mean alpha of .81 with coefficients ranging from .73 to .92. Five other studies had also investigated retest reliability and reported coefficients from .60 to .83.

Validity of the BDI has also been tested thoroughly in a large number of studies both enrolling psychiatric and non-psychiatric samples. About 35 studies had investigated correlational patterns with a variety of concurrent measures of depression (Beck et al., 1988). The meta-analysis revealed satisfactory associations with clinical ratings as well as other self-report measures of depression (e.g. Hamilton Psychiatric Rating Scale for Depression, Zung

Self-reported Depression Scale) indicating good concurrent validity. A number of studies have confirmed discriminant validity of the BDI in differentiating normals and psychiatric patients (see Beck et al., 1988 for review). Construct validity was supported in studies showing associations with biological correlates (e.g. 11-hydroxicorticosteroids), suicide and alcoholism, and measures of anxiety. With respect to factor analytic studies, the BDI appears to be measuring a general second-order syndrome of depression which suggests three interrelated factors reflecting negative attitudes, performance difficulties, and somatic complaints. The composition and number of factors seem to be dependent on the sample being studied.

The various forms of the BDI have been used widely in research and clinical work with both clinical as well as subclinical populations (Endler et al., 1999). A copy of the BDI is provided in the appendix.

3.6.3. Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983)

The Perceived Stress Scale was used to measure the degree to which situations in one's life are appraised as stressful. According to Cohen et al. (1983), its items were selected to tap the degree to which respondents found their lives unpredictable, uncontrollable, and overloading. The scale also includes a number of direct queries about current levels of experienced stress. The PSS is an economical scale that can be administered in only a few minutes and is easy to score. The authors note that because levels of appraised stress should be influenced by daily hassles, major events, and changes in coping resources, the predictive validity of the PSS is expected to fall off rapidly after 4 to 8 weeks.

For each of the 14 PSS items, ratings of distress experienced "in the past week including today" were obtained from the participants. Answers to the PSS are given in a Likert-type format ranging from 0 (never experienced) to 4 (very often experienced). Responses to single items are summarized so that the total score has a potential range from 0 to 56.

The PSS was validated in three samples of healthy volunteers, including two college samples (n=332 college freshmen and n=114 from an introductory personality psychology class, respectively) and 64 participants of a community smoking-cessation program. Internal consistency of the scale ranged from .84 to .86 in the three samples tested. As hypothesized, retest reliability was high after a two-day interval (r_{tt}=.85) but low after six weeks (r_{tt}=.55). The PSS was found to be correlated in the expected manner with a range of self-report (e.g. life events) and behavioral criteria (utilization of health services), supporting its concurrent

and predictive validity. The PSS, although highly correlated with depressive symptomatology, was found to measure a different and independently predictive construct (Cohen et al., 1983). In summary, the PSS is a brief and easy-to-administer measure of perceived stress. It has been proven to possess substantial reliability and validity. The authors suggest it as a potential tool for examining issues of appraised stress levels in the etiology of disease and behavioral disorders. The PSS as used in the study is provided in the appendix.

3.6.4. Brief Symptom Inventory (BSI; Derogatis & Spencer, 1982).

The Brief Symptom Inventory is a 53-item self-report symptom inventory that was developed from its longer parent instrument, the SCL-90-R (Derogatis et al., 1976). It is designed to assess the psychological symptom status of psychiatric and medical patients, as well as individuals who are not patients (Derogatis & Melisaratos, 1983). The items were selected to reflect best these primary symptom dimensions of the SCL-90-R in a brief measurement scale. In addition to these dimensions, there are three global indices of distress associated with the BDI: The General Severity Index (GSI), the Positive Symptom Index (PSDI), and the Positive Symptom Total. The function of each of these global measures is to communicate in a single score the level or depth of symptomatic distress currently experienced by the individual. The GSI is the single best indicator of current distress levels. The authors suggested it should be utilized in most instances where a single summary measure is required. The PSDI is a pure intensity measure while the PST is simply a count of the symptoms which the participant reports experiencing to any degree. In the study presented here, only the GSI was therefore used.

Each item of the BSI is rated on a 5-point Likert scale (0-4) ranging from “not-at-all” to “extremely”. Subjects indicated to what extent each of 53 symptoms caused them discomfort "*in the past week including today*". The authors report an approximate completion time of less than 10 minutes. The BSI is divided into nine subscales (somatization, obsessive, compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism). Validity and reliability have been established in three different samples: Psychiatric out-patients (n=1002), psychiatric in-patients (n=310), and non-patients (n=719). Internal consistency alpha coefficients ranged from .71 to .85. Test-retest reliability coefficients ranging from .68 to .91 for the symptom dimensions and a coefficient of .90 for the GSI.

Convergent and discriminant validity of the BSI was established by examining the pattern of correlation coefficients. From the general finding of high convergence for the dimensions of

the BSI with the respective MMPI subscales, the authors infer that the reduction of length of the SCL-90-R dimensions had not had a significant effect upon their validity. Factor analysis reproduced the hypothesized factor structure with good agreement, thus supporting the construct validity of the instrument. A copy of the BSI is attached (see appendix).

3.7. Physiological parameters

3.7.1. Cardiovascular measures

Diastolic (DBP) and systolic blood pressure (SBP) as well as heart rate (HR) were monitored before (baseline) and during the stress tasks using an automated monitoring device (Spacelabs model 90207). Readings for DBP, SBP, and HR were obtained at two minute intervals for the entire habituation period and during the stress test.

Baseline levels for SBP, DBP, and HR were calculated by averaging the three readings taken for each measure at the end of the habituation period (i.e. covering the 6 minutes before stressor onset). High correlations between measures taken during habituation period as well as between the measures taken during the two tasks (ranging from 0.68 to 0.86, all *p*s < 0.001) were found. Thus, mean SBP, DBP, and HR values were calculated by averaging measures taken during the two tasks to increase reliability (Kamarck, 1992). Cardiovascular measures were used to test the effectiveness of the stressor in the sample.

3.7.2. Endocrine assays

catecholamines

Epinephrine and norepinephrine plasma concentrations were measured using commercial radioenzymatic catecholamine assaykits (Amersham Pharmacia Biotech, Inc, Piscataway, NJ 08855). Catecholamines in plasma samples were converted to [3H]O-methylated derivatives by treating aliquots of plasma with catechol-O-methyltransferase (COMT) and S-adenosyl-L-[methyl-3H]methionine; the [3H]O-methylated derivatives were separated by thin-layer chromatography on silica-gel plates and spots corresponding to norepinephrine and epinephrine were scraped off the plates and counted in a liquid scintillation counter. This assay is highly specific for epinephrine and norepinephrine, respectively. The sensitivity of this assay is in the range 2-5 pg for norepinephrine and epinephrine.

Cortisol

Plasma cortisol levels were assayed with a commercial radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA 90045). 25 • l of calibrator, standards and patient samples was added to labeled cortisol-Ab coated tubes. 1.0 ml of ^{125}I cortisol was added and the tubes were vortexed. After 45 minutes incubation in a water bath at 37C, tubes were decanted and samples were counted for 1 minute on a gamma counter. This assay is highly specific to cortisol with very low crossreactivity to other compounds that may be present in patient samples. Intra assay coefficient of variation (CV) was 2.4% and inter assay CV 7.4%. The detection limit of this assay is 0.2 • g/dl.

3.8. Hypotheses

While considerable evidence from studies with large sample sizes suggests that women at increased familial risk of breast cancer experience increased levels of psychological distress, only a very limited number of studies have to date investigated physiological parameters. The study presented here is the first effort to test acute endocrine stress responses in this population.

The main hypothesis of this work is that women at increased familial breast cancer risk exhibit altered endocrine stress responses. It was further hypothesized that this phenomenon can be explained by the heightened levels of psychological distress in this population. In other words, a mediating effect of psychological distress on the relation between familial risk of cancer and endocrine stress responses was postulated.

Four sets of hypotheses were formulated. For quick reference, the corresponding pathway in the biobehavioral model by Bovbjerg and Valdimarsdottir is mentioned with every set of hypotheses.

1. FR+ is associated with altered endocrine responses (Pathway A-C)

Women at higher breast cancer risk were expected to exhibit alterations in the endocrine stress responses to the standardized laboratory stressor in acute reactivity and / or changes in the recovery pattern after termination of the stressor. Little is known about endocrine parameters in women at increased familial breast cancer risk. The literature on chronic stress and acute endocrine responses seems to suggest that increased reactivity and / or delayed recovery are likely to occur. However, the theoretical model of allostatic load proposes that both increased as well as inadequately weak stress responses can have deleterious long term

effects. Thus, “alterations” in endocrine responses were postulated, i.e. all hypotheses were tested two-sided.

2. FR+ is associated with increased distress (Pathway A-B)

Based on the literature (see section 2.1.3.), increased levels of stress and depressive symptomatology are expected in women at familial risk of breast cancer compared to women at normal risk. FH+ women are expected to have significantly higher scores on the Perceived Stress Scale (PSS), the General Severity Index (GSI) of the Brief Symptom Inventory (BSI), and the Beck Depression Inventory (BDI) when compared to women at normal risk.

3. Increased distress is associated with altered endocrine responses (Pathway B-C)

Integrating findings from animal and human studies that have shown alterations of the endocrine stress responses in individuals with prior exposure to chronic stress, it was expected to find changes of catecholamine and cortisol responses to the laboratory stressor (see section 2.3.) in women with higher levels of psychological distress and depressive symptoms.

4. Increased distress mediates the association between FR+ and endocrine response (Pathway A-B-C)

It was expected that higher levels of stress-related measures hypothesized in the FR+ group would mediate alterations in acute endocrine stress response. A mediation effect requires that hypotheses 1., 2., and 3. are confirmed,. Also, statistically controlling for the measures of psychological distress eliminated or at least reduce the association of familial breast cancer risk and endocrine stress responses (see Statistical Analysis below).

3.9. Statistical analysis

A step-wise statistical analysis was conducted in order to test the proposed association with the modified biobehavioral model of Bovbjerg and Valdimarsdottir (2001). In the first step (3.9.1.), potentially confounds were examined. Since the study sample was relatively small, only variables that differed significantly between groups were statistically controlled for in the subsequent analysis in order to increase statistical power. Sections 3.9.2. through 3.9.5. directly correspond to the four sets of hypotheses described in section 3.8.

The endocrine parameters were tested for outliers and values more than three standard deviations from the mean were excluded. For all tests of endocrine stress response, repeated measures analyses of variance were computed. This approach was preferred to linear regression models with area under the curve as the dependent variable, because it takes the dynamic of the response into account. Furthermore, Corder-Bolz (1978) has shown that ANOVA produces the most reliable results in a repeated measures design compared to other models. It is furthermore robust in case the groups differ in their baseline levels.

3.9.1. Control of confounds and test of stressor effectiveness

3.9.1.1. Group differences in background variables

Differences in categorical background variables (ethnicity, marital status, living arrangement, education, employment status, and income) were tested using chi-square statistics or Fisher's exact test for small cell number when appropriate.

3.9.1.2. Group differences in control variables

Group differences in control variables (age, weight, height, BMI, menstrual cycle, nicotine, caffeine, and alcohol intake, sleep) were examined using one-way analysis of variance (ANOVA) with group as the between-subjects factor (FR- control vs. FR- stress vs. FR+ stress) and the respective measure as the dependent variable. In case of significant F values, Bonferroni-adjusted post-hoc tests were performed. Differences in categorical variables (e.g. obese vs. non obese, based on BMI classification mentioned in section 3.5.) were tested using chi-square statistics or Fisher's exact test for small cell number when appropriate.

3.9.1.3. Baseline differences in physiological measures

Baseline differences in cardiovascular variables as well as endocrine parameters (epinephrine, norepinephrine, cortisol) were evaluated using one-way ANOVAs with group as the between-subjects factor (FR- control vs. FR- stress vs. FR+ stress). In case of a significant F value, Bonferroni-adjusted post-hoc tests were performed.

3.9.1.4. Effectiveness of the stressor

In order to test whether the stress test elicited significant acute stress response in our hands, cardiovascular and self-report VAS data were analyzed using a one-way ANOVA with group as the between-subjects factor (FR- control vs. FR- stress vs. FR+ stress) and changes from baseline as the dependent variable. To reduce Type I error, Bonferroni-adjusted between groups post hoc tests were computed to explore the source of significance.

3.9.2. Familial risk of breast cancer: Endocrine reactivity and recovery patterns (HYPOTHESIS 1)

To test the first set of hypotheses, endocrine acute responses and recovery curves throughout the post-stress period were examined using repeated measures ANOVA with group as the between-subjects factor (FR- control vs. FR- stress vs. FR+ stress) and changes from baseline as the within-subject factor. To reduce Type I error, Bonferroni-adjusted between-groups post hoc tests were computed for each time point separately, to explore the source of significant interaction terms.

3.9.3. Levels of psychological distress in women at familial risk (HYPOTHESIS 2)

To investigate whether women with or without heightened breast cancer risk differed with respect to the self-report questionnaire data, t-tests for independent samples were computed using FR+ vs. FR- as the grouping factor. This was performed for the BDI score, the GSI of the BSI, the PSS, as well as the averaged VAS baseline score.

3.9.4. Alterations of endocrine stress responses in women with high levels of psychological distress (HYPOTHESIS 3)

To examine endocrine responses induced by the stress test, delta values (change compared to baseline) were computed for all post stressor time points, thus reducing the impact of well known interindividual variability in basal levels. For a preliminary analysis of the association between the psychological variables and endocrine stress responses, Pearson correlation coefficients were computed for delta scores (change from baseline) in the three endocrine measures with the BDI, GSI, PSS, and VAS baseline. As mentioned above, an repeated measures ANOVA approach was used to examine the dynamic of the stress response. Furthermore, this approach allows a better statistical evaluation of reactivity as well as recovery patterns. To obtain distinct groups, median splits were performed on the four psychological measures of distress. This grouping factor was then used as the between-subjects factor in Time x Group ANOVAs (repeated measures), where Time was entered as the within-subject factor.

An incomplete factorial design was used for the ANOVAs. In order to increase statistical power, the median splits were not performed in the non-stressed control group (control FR-). It was hypothesized that the stress response would differ in group of high vs. low scores on the distress measures. However, no effects of different levels of psychological distress on resting levels of endocrine measures over time were expected. That is, no different patterns of

cortisol and catecholamines were expected in women with high vs. low distress levels who were not subjected to the stressor (control group). In order to test this assumption, median splits were also performed in the non-stressed control group and repeated measures ANOVAs were run similarly to the approach mentioned above. In case of non-significant Time x Group interaction in this analysis, the assumption was considered to be valid and all control subjects were collapsed into one no-stress group.

3.9.5. Mediation of FR effects by depressive symptoms, perceived stress and VAS baseline (HYPOTHESIS 4)

Based on the modified biobehavioral model of Bovbjerg and Valdimarsdottir (2001) and the empirical evidence from the chronic stress / acute stress literature reviewed above, it was assumed that increased levels of psychological distress in FR+ women would (in part) account for alteration in the endocrine stress response hypothesized in this group.

Baron and Kenny (1986) have pointed out the importance of not using the terms "moderator" and "mediator" interchangeably. Here, a moderator variable is defined as a "third variable, which partitions a focal independent variable into subgroups that establish it's domain of maximal effectiveness in regards to a given dependent variable". A mediator however is a third variable, "which represents the generative mechanism through which the focal independent variable is able to influence the dependent variable of interest" (p. 1173).

A given variable may be said to function as a mediator to the extent that it accounts for the relation between the predictor and the criterion. As Baron and Kenny put it, "...moderator variables specify when certain effects will hold, mediators speak to how and why such effects occur" (p. 1176). A variable functions as a mediator if the following three conditions are met: 1. Variations in levels of the independent variable (e.g. family history) significantly account for variations in the presumed mediator (measures of distress and depressive symptoms), 2. Variations in the mediator account for variations in the dependent variable (endocrine stress responses), and 3. statistically controlling for the mediator would lead to a previously significant relation between the independent and the dependent variable no longer being significant.

All analyses were conducted with statistical software (SPSS 10.0), with a p value of less than .05 considered significant.

RESULTS

4.1. Control for confounds, test of stressor effectiveness

4.1.1. Background variables: Demographic characteristics of the study sample

Chi squared tests revealed no differences on demographic variables: employment (chi square=10.4; p=.74), level of education (chi square=3.0; p=.80), family income (chi square=12.2; p=.28), marital status (chi square=8.6; p=.20), or ethnicity (chi square=11.7; p=.17). Detailed distributions are given in table A in the appendix.

4.1.2. Group differences in control variables

In order to control for potential confounds, group differences in control variables that are known to potentially influence the endocrine system were tested.

The groups did not differ significantly in any of the control variables: Age (ANOVA F(2, 77)=.67; df=2; p=.52), height (ANOVA F(2, 76)=.80; p=.45), weight (ANOVA F(2, 74)=2.7; p=.08), body mass index (BMI) (ANOVA F(2, 72)=1.29; p=.29), or days since last period (ANOVA F(2, 75)=.51; p=.60). Means and standard deviations for these variables are shown in table 2.

Table 2: Control variables of the study sample divided into FR- Control, FR- Stress, and FR+ Stress (mean $\pm$ standard deviation).

Variable	FR- Control	FR- Stress	FR+ Stress
Age (years)	33.6 $\pm$ 9.4	35.8 $\pm$ 8.2	35.9 $\pm$ 7.4
Height (cm)	163.6 $\pm$ 7.5	165.8 $\pm$ 5.9	164.8 $\pm$ 7.3
Weight (kg)	59.2 $\pm$ 6.9;	64.9 $\pm$ 11.7	61.4 $\pm$ 8.8
Body Mass Index (BMI)	22.3 $\pm$ 2.9	23.7 $\pm$ 3.8	22.6 $\pm$ 3.4
Days since last period	12.2 $\pm$ 11.6	16.4 $\pm$ 21.2	15.5 $\pm$ 14.3

Fisher's Exact Test two-sided were used for categorical data with small cell sizes showed that there were no significant group differences in the distribution of women having a BMI greater

than 30, i.e. WHO obesity class I (FR- control one subject; FR- stress three subjects; FR+ stress one subject; Fisher's Exact Test two-sided significance p=.65).

Thus, none of these variables was statistically controlled for in the following analyses. As there was a trend for a group difference in body weight, all the analyses described below were also conducted with weight included as a covariate and an identical pattern of significant results was seen. Furthermore, weight was not significantly correlated with any of the endocrine parameters at any time point (all coefficients below .17).

Descriptives for alcohol, caffeine, and nicotine intake as well as hours of sleep are displayed in the appendix (tables B and C) for the three days prior to the stress session (visit 2). As shown in the tables, there were no significant differences for hours of sleep, nicotine, alcohol, and caffeine consumption except for consumption of alcoholic beverages the night before the experiment (further analysis of this, see below).

Self-reported compliance with the instructions regarding alcohol and caffeine intake were satisfactory. Only one subject reported to have had 3 alcoholic drinks the night before the experiment, while the vast majority had none (81%). Alcohol consumption the night before the experiment was not significantly correlated with any of the endocrine measures at any time point (all coefficients below .14). Regarding caffeine intake, seven subjects (9%) reported that they had consumed 2 cups of coffee while 53% had none and 38% had one cup. The subjects with 2 cups each were equally distributed within the three groups (Fisher's Exact Test two sided significance p=.41). Again, caffeine intake the morning of the experiment was not significantly correlated with epinephrine, norepinephrine, or cortisol levels at any of the time points (all coefficients below .17). Thus, none of these variables was statistically controlled for in the analyses below.

4.1.3. Baseline levels of endocrine measures

To investigate baseline differences in the endocrine variables, one-way ANOVAs were conducted (see table 3 for group mean levels). No significant baseline differences between groups were seen in plasma cortisol (ANOVA $F(2, 80)=1.3$; $p=.27$), epinephrine (ANOVA $F(2, 80)=1.4$; $p=.26$), or norepinephrine (ANOVA $F(2, 80)=.09$; $p=.92$).

Table 3: Baseline values in epinephrine, norepinephrine, and cortisol of the study sample divided into FR- Control, FR- Stress, and FR+ Stress (mean ± standard deviation).

Variable	FR- Control	FR- Stress	FR+ Stress
Epinephrine [pg/ml]	112.6 ± 109.8	72.8 ± 101.1	66.8 ± 64.4
Norepinephrine [pg/ml]	331.3 ± 224.7	356.2 ± 283.3	344.2 ± 136.9
Cortisol [µl/dl]	15.4 ± 11.4	13.1 ± 4.7	11.6 ± 4.9

4.1.4. Effectiveness of the stressor

Effectiveness of the stressor was tested using delta scores (post-pre) for self-report VAS and cardiovascular data. Group differences were tested by computing one-way ANOVAs. Significant group differences in self-reported distress (VAS) responses to the stress test were observed (ANOVA $F(2, 79)=63.0$; $p<.001$). Analysis of variance also revealed significant group effects for HR reactivity during the stress test (ANOVA $F(2, 79)=23.89$; $p<.001$) as well as systolic blood pressure (SBP) (ANOVA $F(2, 79)=25.84$; $p<.001$) and diastolic blood pressure (DBP) (ANOVA $F(2, 79)=12.95$; $p<.001$). Post-hoc tests showed significant HR differences between all three groups, with a significantly stronger stress response in FR+ subjects compared to FR- subjects, while both stress groups significantly differed from unstressed controls. For VAS, DBP and SBP, both groups undergoing the stress tests showed significantly elevated delta scores above control group levels but did not differ from each other (see figure 4 and 5). Confirmatory analyses of raw data revealed an identical pattern of significance as that seen with delta values.

4.2. Familial risk of breast cancer and endocrine stress response

To investigate the third set of hypotheses, endocrine responses were tested in women with (FR+ stress) or without (FR- stress) family histories of breast cancer compared to unstressed controls (FR- control). As mentioned above, delta values were used to control for the considerable variance between individuals in the endocrine parameters. It should be noted that analysis using absolute concentrations yielded the exact same pattern of significance. Absolute levels for the three groups at all time points can be found in the appendix (table D).

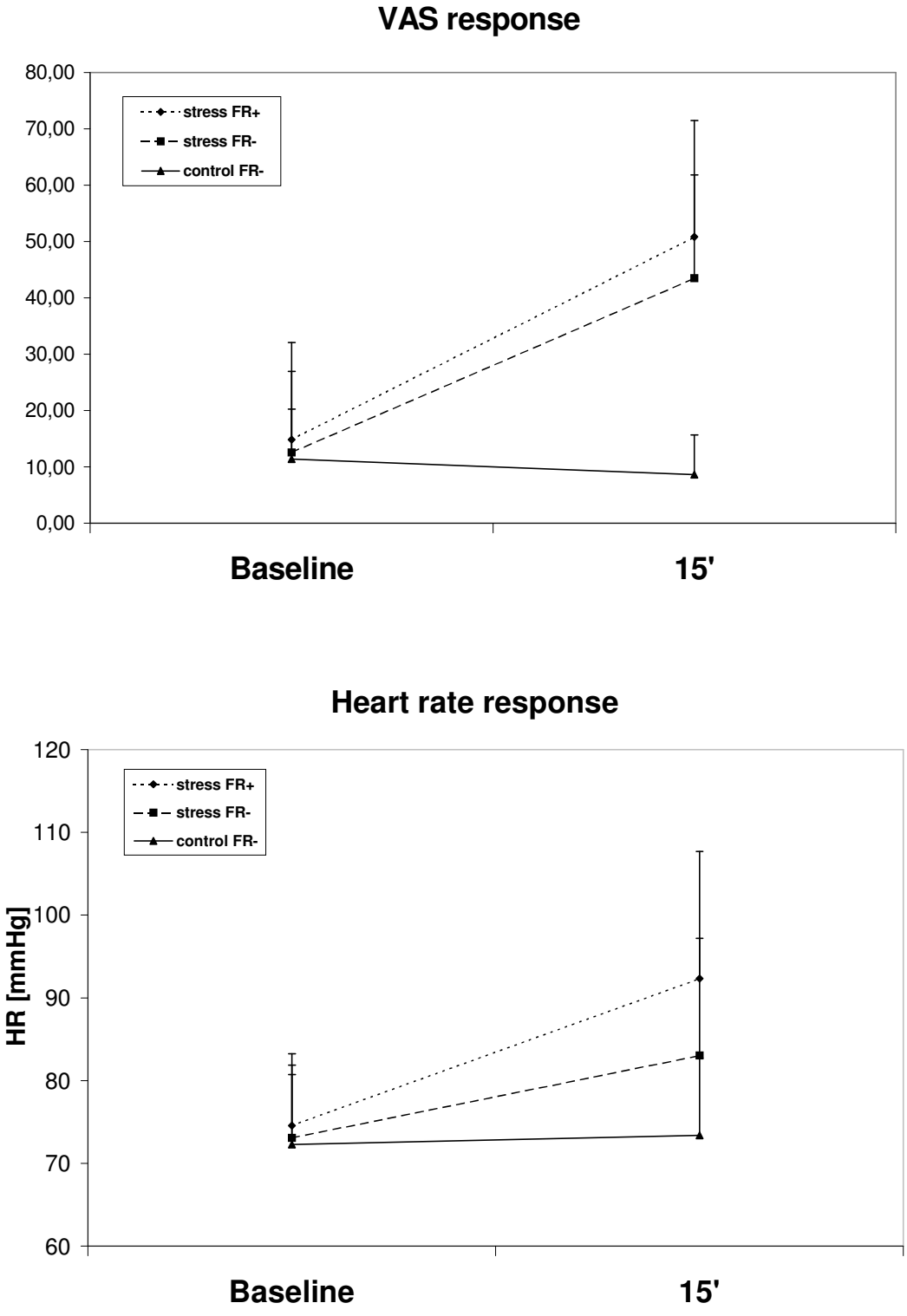

Figure 4: Response patterns of self-reported mood (VAS), and heart rate (HR) in FR- control, FR- stress, and FR+ stress women. VAS are average score (means $\pm$ SD) for the ten analog scales obtained directly before (baseline) and after (15') the stressor. HR values (means $\pm$ SD) are given for baseline and average of readings during the stressor (15'). See text for details.

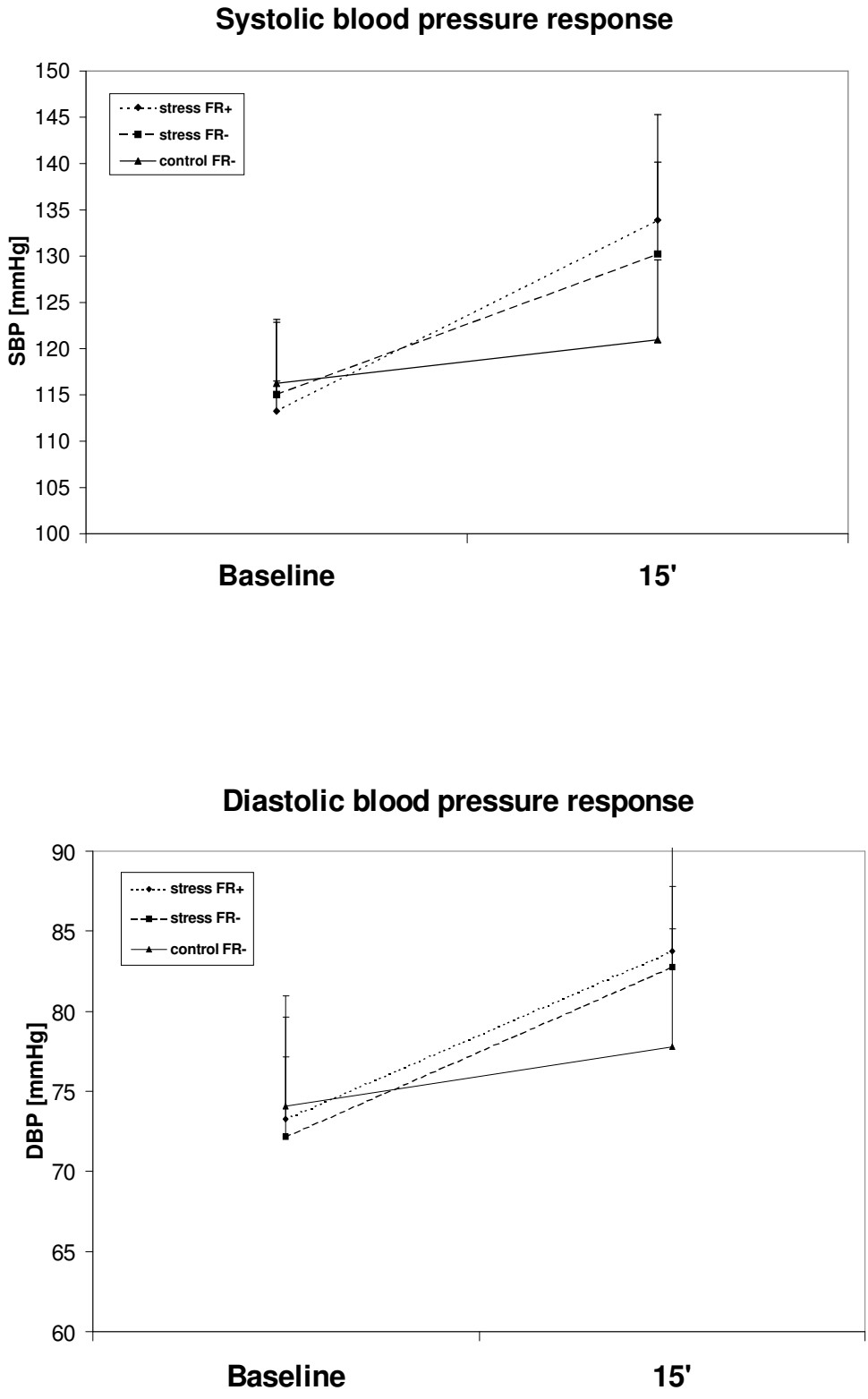

Figure 5: Response patterns of systolic blood pressure (SBP), and diastolic blood pressure (DBP) in FH- control, FH- stress, and FH+ stress women. Values (means $\pm$ SD) are given for baseline and average of readings during the stressor (15'). See text for details.

A marked acute stress response in epinephrine was observed in both stressed groups, which began to resolve at 30 min post baseline in FR- but not in FR+ women (see figure 6). Multivariate tests in repeated measures ANOVA showed a significant Group main effect (F(2,

80)=5.20; p=.01), no main effect for Time (F(3, 78)=.46, p=.70), but a significant Group x Time interaction (F(6, 158)=2.64, p=.02). At 15 min post baseline, epinephrine was significantly higher in both stress groups compared to the Control Group. At 30 min, only the FR+ Stress Group remained significantly higher than controls as indicated by significant Bonferroni-adjusted post-hoc tests (see figure 6). Although the pattern of responses seen in figure 6 is suggestive of reductions in epinephrine levels in the control group, single group ANOVAs revealed significant Time effects only for the FR+ stress group (F(3, 14)=4.29; p=.02), while there were no significant Time effects for either of the other two groups (FR- control F(3, 27)=1.92; p=.15; FR- stress F(3, 33)=.93; p=.44).

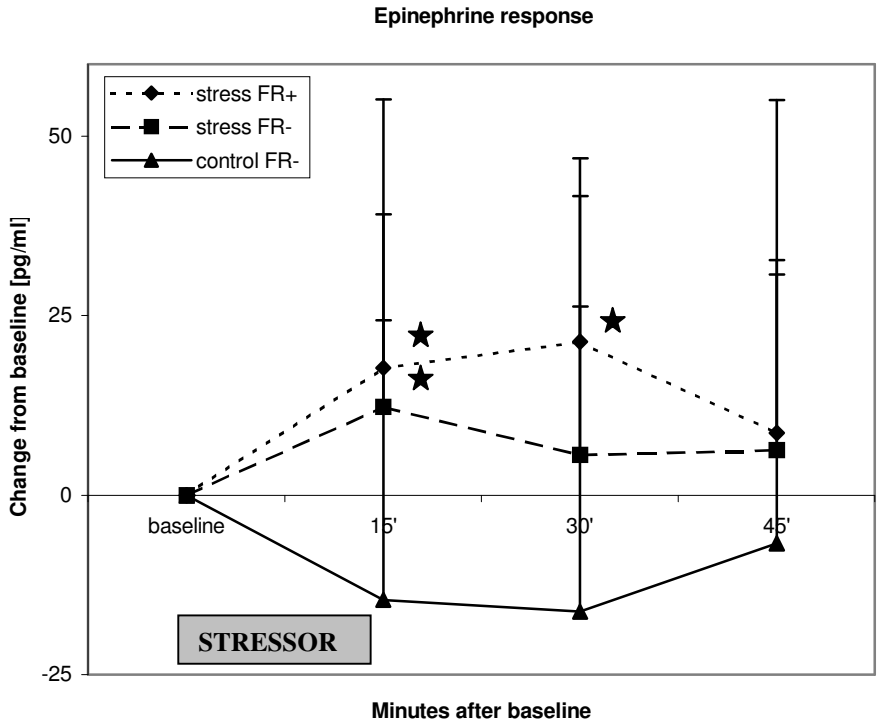

Figure 6: Epinephrine response (means ± SD) to an acute psychological stressor in healthy women at familial risk (stress FR+, n=17) and normal risk for breast cancer (stress FR-, n=36) compared to non-stressed women at normal risk (control FR-, n=30). Astrixes indicate significant post-hoc difference (Bonferroni adjustment) to unstressed control group.

Responses of plasma norepinephrine levels showed no significant Group (F(2, 80)=.01, p=.99), Time (F(3, 78)=1.10, p=.36), or interaction effects (F(6, 158)=1.28, p=.27). Delta scores for norepinephrine are graphically presented in figure 7.

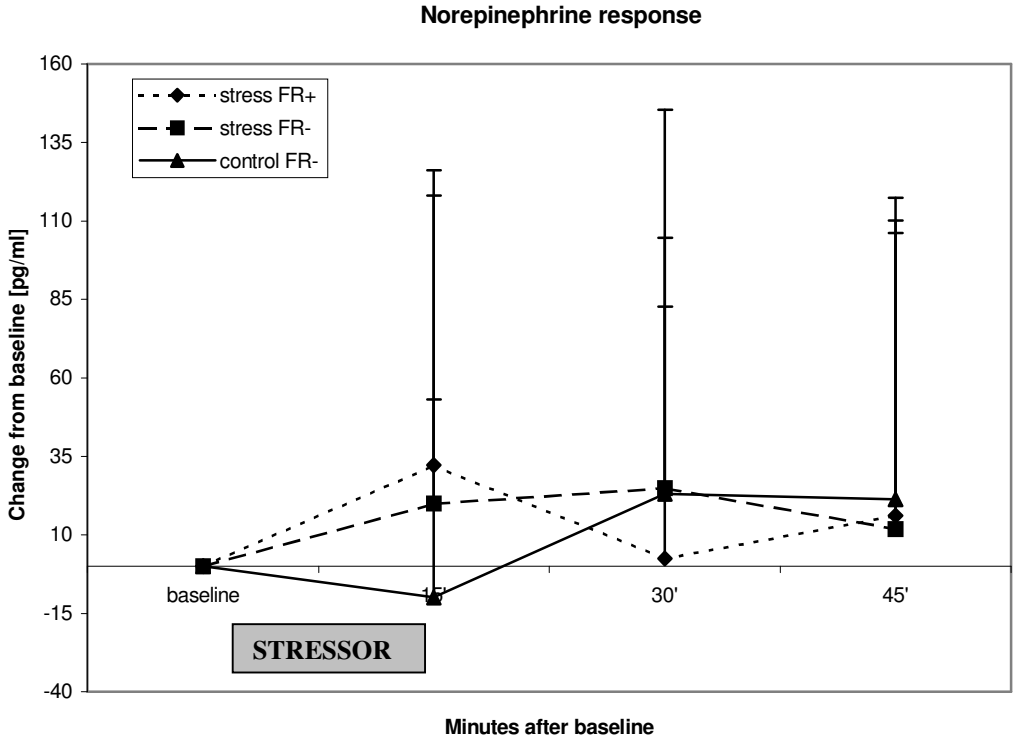

Figure 7: Norepinephrine response (means ± SD) to an acute psychological stressor in healthy women at familial risk (stress FR+, n=17) and normal risk for breast cancer (stress FR-, n=36) compared to non-stressed women at normal risk (control FR-, n=30).

Women at familial risk for breast cancer showed an increase in plasma cortisol levels after the stress test while women at normal risk in the stress group as well as the control condition declined (see figure 8). As would be expected during the mroning hours, cortisol levels declined in the non-stressed control group. Multivariate tests showed significant Time (F(3, 78)=16.57, p<.001) and Group (F(2, 80)=3.82, p=.03) main effects as well as a significant Group x Time interaction (F(6, 158)=2.29, p=.04) for cortisol. Cortisol levels of the FR+ Stress Group remained significantly elevated above mean values for the Control Group until

30 min post baseline while the FR- Stress Group did not differ from control subjects at any time point (Bonferroni-adjusted post-hoc tests, see figure 8).

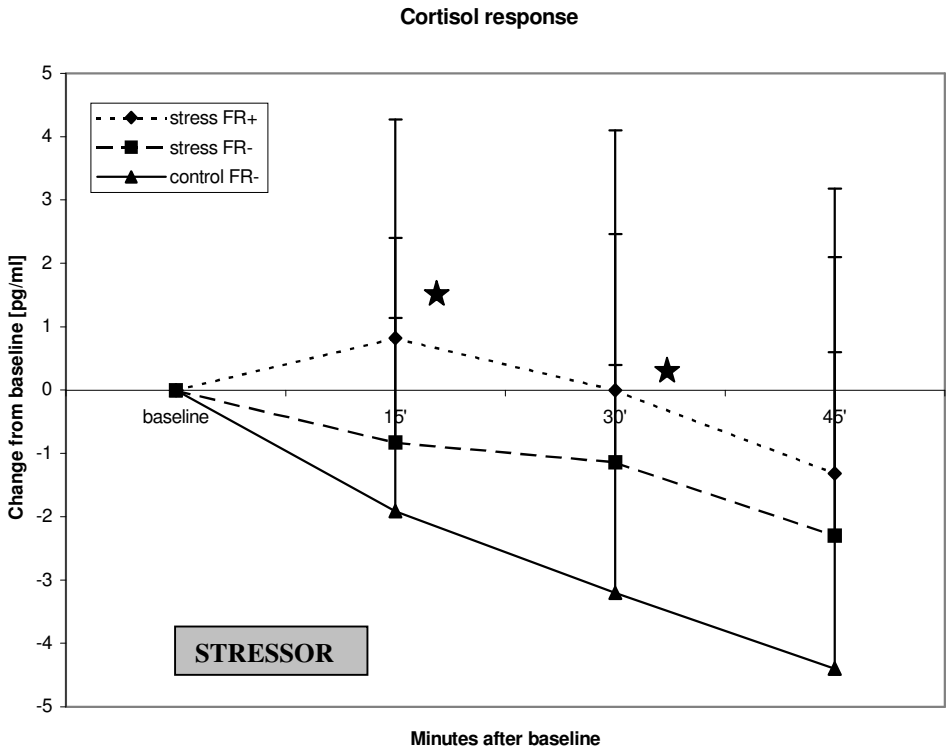

Figure 8: Cortisol response to an acute psychological stressor in healthy women at familial risk (stress FR+, n=17) and normal risk for breast cancer (stress FR-, n=36) compared to non-stressed women at normal risk (control FR-, n=30). Asterix indicates significant post-hoc difference (Bonferroni adjustment) to unstressed control group.

4.3. Group differences in self-report measures: BDI, GSI, PSS, VAS

In order to test whether women with or without increased familial risk of developing breast cancer differed with respect to scores on the psychological self-report measures, t-tests for independent samples were computed. Mean scores and standard deviations are shown in table 4. While mean scores were slightly higher in FR+ women in the BDI, no significant group differences between FR- and FR+ women were seen in any of the questionnaires used.

Table 4: Group differences in psychological self-report measures.

	FR+ vs. FR-	N	Mean ± SD	t	df	p
GSI	FR-	63	0.47 ± .48	-0.54	78	.59
	FR+	17	0.54 ± .05			
PSS	FR-	63	21.38 ± 7.73	-0.68	78	.50
	FR+	17	22.82 ± 7.91			
BDI	FR-	63	5.79 ± 5.75	-1.35	78	.18
	FR+	17	8.24 ± 9.09			
VAS baseline	FR-	63	12.04 + 12.16	-0.77	78	.45
	FR+	17	14.85 + 17.20			

GSI: General Severity Index; PSS: Perceived Stress Scale; BDI: Beck Depression Inventory; VAS: Visual Analog Scale (sum score).

4.4. Associations of endocrine responses with self-report measures

In order to determine the associations of self-report measures and endocrine responses to the stress test, Pearson bivariate correlations were computed using delta scores in the endocrine measures. Correlations were run for each time point separately. The correlation coefficients for continuous as well as dichotomized distress measures and endocrine parameters are displayed in the appendix (tables E, F, G).

Raw scores of self-report measures were not significantly correlated with endocrine stress response. In order to compute repeated-measures ANOVAs (see section 3.9.), median splits were computed. The dichotomized variables (based on BDI, GSI, PSS, and VAS baseline), moderately (but significantly) correlated with endocrine measures. Directly post stressor termination, all four scales were positively correlated with delta values in epinephrine and cortisol, indicating that higher scores were associated with increased stress reactivity. At 30 minutes post baseline, these associations remained significant. However, at 45 minutes post baseline, only BDI was still positively correlated with both epinephrine and cortisol. These positive correlations with endocrine post-stressor levels are suggestive of a delayed recovery in women scoring in the upper half of the requisite scales.

Repeated measures ANOVAs were computed using the median split variables (upper half vs. lower half) as the between-subjects factor. As mentioned above, delta scores of endocrine parameters (change from baseline) were used. Absolute values can be found in the appendix (tables H, I, J, K). To estimate the impact of distress independent of family history of breast

cancer, FR was statistically controlled for in all analyses. Since we were interested in the association of self-report scores and stress response patterns, median splits were computed in a subsample only comprising the subjects undergoing the stressor (see Methods section).

Test of assumption for incomplete factorial design: Median splits in control subjects not undergoing the stress

However, to make sure that these measures were not differentially associated with an altered endocrine pattern under resting conditions (control group), we performed median splits on all measures for the control group as well. A Time x Group ANOVA was then run in the control group as well using the median split variable as the between-subjects factor.

For none of the four parameters, a significant Time x Group interaction was seen on resting (control group) levels. Results from the computed ANOVAs and significance levels can be found in the appendix (tables L, M, N).

Since no differential effects on resting endocrine levels were seen based on BDI, PSS, GSI and VAS, the following analyses were run with all control subjects collapsed into one group in order to increase statistical power.

Group differences in control variables after median splits

VAS baseline: The median split on levels of baseline VAS summary scores led to subject numbers of n=26 in the VAS low and VAS high group, respectively. As seen with the other grouping parameters, no differences emerged in the three group comparison (VAS high vs. VAS low vs. Controls) for the variables ethnicity (χ^2=6.58; p=.58), marital status (χ^2=12.21; p=.06), education (χ^2=4.13; p=.66), employment (χ^2=10.40; p=.73), and income (χ^2=9.23; p=.51). Groups did also not significantly differ in age (F(2; 76)=1.44; p=.24) and BMI (F(2; 71)=.61; p=.55).

GSI: After computing a median split, the stress groups were divided into a GSI low (n=25) and a GSI high group (n=27). Unequal subsample sizes are due to ties in the score distribution, which were assigned to the GSI-high group based on the distribution characteristics. No significant group differences between controls, GSI-high and GSI-low subjects were observed in the background variables ethnicity (χ^2=9.60; p=.30), marital status (χ^2=8.10; p=.23), education (χ^2=4.45; p=.62), employment (χ^2=8.26; p=.88), and income (χ^2=6.31; p=.79). There were also no differences in age (F(2; 76)=1.64; p=.20) or BMI (F(2; 71)=.61; p=.55).

PSS: Subjects scoring below (n=27) and above (n=25) the median on the PSS, as well as controls were compared with respect to the background variables. Unequal subsample sizes are due to ties in the score distribution, which were assigned to the PSS-low group based on the distribution characteristics. Again, no significantly different distributions were found for ethnicity (χ^2=11.93; p=.15), marital status (χ^2=8.87; p=.18), education (χ^2=4.95; p=.55), employment (χ^2=9.70; p=.78), and income (χ^2=7.14; p=.41). Mean scores in age (F(2; 76)=.55; p=.58) and BMI (F(2; 71)=.61; p=.55) were also not significantly different.

BDI: The subjects who underwent the stress tests were divided by median split (based on their score on the Beck Depression Inventory; median=5.0) into a BDI-high (scoring above the median n=25) or BDI-low group (scoring below the median n=27). Unequal subsample sizes are due to ties in the score distribution, which were assigned to the BDI-low group based on the distribution characteristics. There were no significant differences in distributions of ethnicity (χ^2=6.63; p=.16), education (χ^2=6.06; p=.11), income (χ^2=4.88; p=.43), employment (χ^2=3.35; p=.65) or marital status (χ^2=3.83; p=.28) between controls, BDI-high and BDI-low subjects. No group differences were found in age or BMI between groups (age F(2, 76)=.59; p=.56; BMI F(2; 71)=1.67; p=.20).

VAS and endocrine responses

The analyses of VAS baseline median split associations with epinephrine response yielded no significant Time (F(3, 75)=.52; p=.67), or Group x Time interaction effect (F(6, 152)=1.54, p=.17). There was however a significant Group main effect (F(2, 77)=3.24; p=.04).

Similarly, no significant Time effect was detected for norepinephrine (F(3, 75)=1.53; p=.21). There was also no significant effect for Group (F(2, 77)=1.31; p=.28) or Group x Time interaction (F(6, 152)=.83, p=.55).

For cortisol, there was a significant Time effect F(3, 75)=10.74; p<.001) but no significant effects for Group (F(2, 77)=1.85; p=.16) or Time x Group interaction (F(6, 152)=1.66, p=.14).

GSI and endocrine responses

The analyses of GSI median split associations with epinephrine response yielded no significant Time (F(3, 75)=.53; p=.66), Group (F(2, 77)=.05; p=.82), or Group x Time interaction effect (F(6, 152)=1.6, p=.16).

Similarly, no significant Time effect was detected for norepinephrine (F(3, 75)=1.72; p=.17). There was no significant effect for Group (F(2, 77)=3.77; p=.06) Group x Time interaction (F(6, 152)=1.99, p=.07).

For cortisol, there was a significant Time effect F(3, 75)=10.63; p<.001) but no significant effects for Group (F(2, 77)=1.18; p=.21) or Time x Group interaction (F(6, 152)=1.99, p=.07).

PSS and endocrine responses

When looking at PSS scores and epinephrine response, there was a significant Group effect (F(2, 77)=3.53; p=.03) but no significant effect for Time (F(3, 75)=.49; p=.70) or Time x Group interaction (Time x Group F(6, 152)=1.8, p=.10). No significant effects were seen in the analysis of norepinephrine response patterns (Time (F(3, 75)=1.47; p=.23), Group (F(2, 77)=1.55; p=.23), Time x Group F(6, 152)=.99, p=.43). Again, the main effect for Time was significant for cortisol (Time (F(3, 75)=10.56; p<.001), Group (F(2, 77)=2.17; p=.12), Time x Group F(6, 152)=1.39, p=.22).

BDI and endocrine responses

BDI scores were associated with differences in the pattern of epinephrine stress response. In the three-group approach, a significant Time x Group interaction was found for epinephrine (F(6; 152)=2.2; p=.04). There was no significant effect for Time (F(3, 75)=.82; p=.49) or Group (F(2, 77)=.39; p=.40). Both stress groups showed a marked stress-induced elevation of epinephrine directly post stress (Bonferroni-adjusted post-hoc comparison with controls p=.02 for BDI low; p=.03 for BDI high). However at 30 minutes post baseline, the BDI-low subgroup had returned to basal levels and concentrations were no longer significantly different from those seen in unstressed controls (p=.11). BDI-high women on the contrary exhibited further increased epinephrine levels at 30 minutes, which were still significantly higher than in women not subjected to the stress test (p=.001, see figure 9).

For norepinephrine, no significant effects for Group (F(2, 77)=.88; p=.42), Time (F(3, 75)=1.50; p=.22), or Time x Group interaction (F(6, 152)=.88; p=.54) was found.

When looking at cortisol responses, there was a significant main effect for Time (F(3, 75)=10.50; p<.001) but not Group (F(2, 77)=1.90; p=.15). Furthermore, a significant Time x Group interaction emerged (F(6, 152)=2.91; p=.01). Post-hoc tests indicated that directly post stressor (15 minutes post baseline), only BDI-low subjects had cortisol levels significantly above the control group (p=.04). At 30 minutes post baseline, BDI-high subjects were significantly higher than controls (p=.04) while BDI-low subjects did not significantly differ from controls (p=.06).

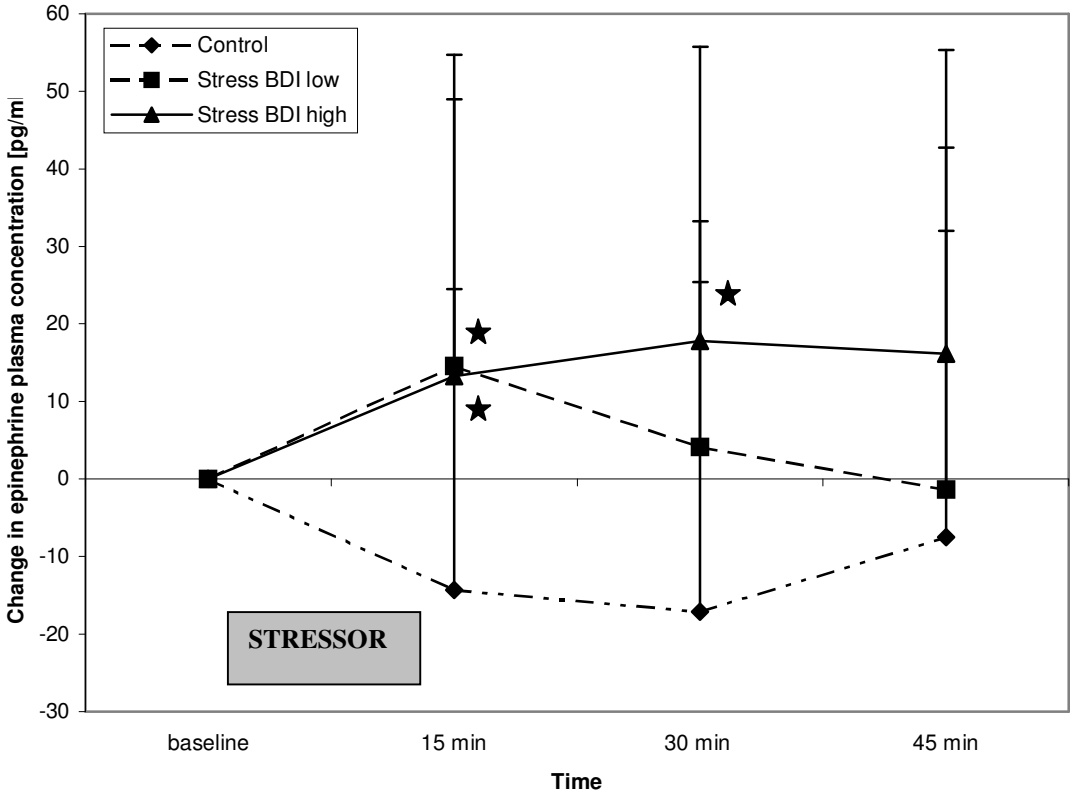

Figure 9: Epinephrine response (mean ± SD) to the stress task in women with high or low BDI scores compared with controls. Levels indicate changes from baseline and are given. Asterix indicates significant post-hoc difference (Bonferroni adjustment) to unstressed control group.

4.5 Mediating effect of psychological variables

The failure to identify a statistically significant association between familial breast cancer risk and self-report measures of distress and depressive symptoms (see above) precluded any investigation of the hypothesized mediating effect of the psychological measures on the relation between FR+ and endocrine stress responses (see Statistical Analysis section). Therefore, no further analyses were run to investigate this hypothesis.

However, from the results presented above it appeared possible that the association between depressive symptoms and alterations of epinephrine response was mediated by increased levels of perceived stress.

Correlation of BDI, PSS, GSI, and VAS baseline

As expected from the literature (e.g. Kuiper et al., 1986; Lovibond & Lovibond, 1995; Pengilly & Dowd, 2000), there were substantial and statistically significant correlations between measures of depressive symptoms, perceived stress, stress symptomatology, and current mood (see table 5).

Table 5: Interrelations of the self-report measures in the sample.

		GSI	PSS	BDI
GSI	r			
	Sig.			
PSS	r	.764		
	Sig.	.000		
BDI	r	.739	.606	
	Sig.	.000	000	
VAS	r	.707	663	.636
	Sig.	.000	.000	.000

Based on the correlation coefficients of self-report data (median split) and endocrine stress response, there were indications that the relationship of BDI and epinephrine response could be mediated by increased levels of perceived stress in women scoring high on the BDI.

Given the considerable correlations between the GSI, PSS, VAS, and BDI on one hand and the differential effect of BDI on epinephrine response to the acute stress challenge on the other as well as the significant correlations of dichotomized GSI, PSS, and VAS with epinephrine levels, a potential mediating effect was explored by using the Baron & Kenny model (Baron & Kenny, 1986, see Methods section).

Repeated measures ANOVA using BDI as the grouping factor while entering GSI, PSS, and VAS basal as covariates were run. Analysis confirmed that the observed differential endocrine stress response in BDI-high vs. BDI-low group was not mediated by differences in baseline GSI, PSS or VAS scores, since none of these covariates showed a significant interaction by time (all $p>.30$) while BDI Group x Time interaction remained highly significant ($F(6; 144)=2.9$, $p=.01$).

5. DISCUSSION

In the study presented here, the association of being at increased familial risk for developing breast cancer and neuroendocrine stress responses were investigated. A standard laboratory stressor was employed, which is widely used in psychobiological research and that has repeatedly been shown to elicit reliable neuroendocrine activation. Based on the literature, it was tested whether potential alterations in acute stress reactivity and recovery are related to increased levels of psychological stress and depressive symptoms in this high risk population. Two of the four sets of hypotheses outlined in section 3.8 were supported by the data obtained: An increased reactivity and / or delayed recovery in women at familial risk was seen for epinephrine and cortisol. A delayed epinephrine recovery was also observed in women with higher scores on the BDI. However, no significant increased levels of psychological distress could be confirmed in FR+ women. This precluded any investigation of the hypothesized mediation effect of psychological distress on the association of FR+ and stress response alterations. The findings of the study are discussed below with respect to the relevant literature. First, the lack of significant differences in self-reported distress and basal endocrine levels will be evaluated with regard to the literature. Second, I will discuss findings of altered endocrine stress responses in FR+ women. The independent contributions of familial breast cancer risk and psychological distress to the dysregulation of epinephrine recovery are then considered. Subsequently, the limitations of this approach are considered. Finally, possible implications of the results for future research are outlined.

5.1. Familial risk of breast cancer: Associations with psychological distress and basal endocrine activity

5.1.1. Psychological distress

In the first set of hypotheses, it was expected that women at increased breast cancer risk would exhibit higher scores in the measures used to assess depressive symptoms (BDI), perceived stress (PSS), stress symptomatology (GSI), and current mood (VAS baseline).

In contrast to most studies enrolling large numbers of women (Kash et al., 1992; Lerman et al., 1993; Baider et al., 2000; Lindberg & Wellisch, 2001; Neise et al., 2001), we were not able to find a significant difference between women with or without increased familial risk of breast cancer. These studies, however, all had sample sizes between 129 (Neise et al., 2001) and 430 (Lindberg & Wellisch, 2001). Interestingly, most significant findings were found on

scales which very specifically assessed cancer-related stress and anxiety. No differences on depression questionnaires are reported in the literature (Lerman et al., 1993; 1994), with the exception of Cohen's study (2002a, see below). To date, only a few studies have used a comparison group in this area of research. Wellisch et al. (1991) did not find significant differences in psychological symptoms between FR+ (n=60) and FR- (n=60) women. Bovbjerg & Valdimarsdottir (1993) also could not find significantly increased levels of psychological distress between the groups (11 FR+, 32 FR-). On the other hand, Valdimarsdottir et al. (1995) found increased levels of both non-specific distress as well as cancer-related intrusive thoughts in 26 FR+ compared to 26 FR- women. Zakowski et al. (1998) (46 FR+ vs. 43 FR-) and Erblich et al. (2000) confirmed these differences with regard to cancer-specific intrusive thoughts. Interestingly, in a relatively large sample of 93 FR+ women compared to 142 women at normal risk, only a subgroup of high risk women who presented for a regular check-up with symptoms were found to have higher levels of general distress as measured by the GSI of the BSI (Gilbrar, 1998).

The lack of group differences in the BDI is in line with results reported by others in larger samples (Lerman et al., 1993; 1994). On the other hand, the sample of the present study (FR+ n=17) might have been too small in order to detect group differences in psychological distress shown in earlier reports. Although we used the same instruments as previous studies (BDI, BSI), the smallest study with positive findings in the literature had an n=26 (Valdimarsdottir et al., 1995), making statistical power a likely explanation for the differences between the studies. This is also supported by the fact that for all self-report measures, FR+ women in our sample had slightly elevated scores compared to FR- women. However, none of these differences was statistically significant. Additionally, Valdimarsdottir et al. (1995) found the highest stress levels when women presented for a mammography, which is a situation that might trigger cancer-related fears especially in women at familial risk. Also, the majority of FR+ women in the study presented here were recruited at cancer surveillance centers. If heightened psychological distress is in fact a barrier to screening adherence as postulated by Bovbjerg and Valdimarsdottir (2001) as well as Rees and co-workers (2001), it is thus conceivable that a selection bias led to recruitment of women with comparably lower levels of distress.

Rees et al. (2001) have suggested that experience with a relative's disease may play a significant role in mediating the extent of psychological distress. Two studies have to date empirically confirmed this hypothesis: Zakowski et al. (1997) reported that FR+ women, whose mother had died from breast cancer, experienced significantly increased intrusive

thoughts. Erblich et al. (2000a) showed that women who had cared for their affected mother had significantly increased cancer-related stress. This effect was strongest in women who experienced both death and caregiving. In the study presented here, the sample size of FR+ women was too small to allow for subgroup analyses with respect to the women's experiences.

Our findings are in disagreement with some studies that investigated effects of other "naturalistic stressors" on self-report distress. This may of course be due to the different nature of stressors such as unemployment (Ockenfels et al., 1995), caregiving (e.g. Cacioppo et al., 2000), or job strain in high school teachers (Benschop et al., 1994). A paradigm extensively used by the group of Kiecolt-Glaser and Glaser is spousal caregiving for Alzheimer's patients. Here, they repeatedly found higher levels of perceived stress in caregivers. This may indicate that caregiving is a stronger stressor compared to being at increased risk of breast cancer. Although the two stressors may have several common characteristics, dementia caregiver burden has been advanced as an all-encompassing term that refers to the financial, physical and emotional effects of caring for an adult with a disabling condition (Dunkin & Anderson-Hanley, 1998). Not all of these may also be experienced by women at familial breast cancer risk. Additionally, Kiecolt-Glaser and Glaser are generally enrolling spousal caregivers, i.e. elderly persons, thus making effects of age a potential confound (mean age in our study: 35 years). Also, it should be noted that Kiecolt-Glaser, Glaser and colleagues usually selectively recruit caregivers and non-caregivers to maximize group differences in depression and perceived stress (e.g. Hadjiconstantinou et al., 2001). This was not the case in the study presented here and would be very difficult to achieve due to the already low number of subjects who score above the cut-off on the Claus score.

5.1.2. Basal endocrine activity

In the data set, no significant differences in endocrine baseline levels were observed between FR+ and FR- groups. Furthermore, no basal endocrine differences were found when comparing women with high vs. low scores on the BDI, the PSS, the GSI or baseline VAS. Studies of baseline levels of glucocorticoids and catecholamines in humans under chronic stress have yielded inconsistent results. Evidence from the animal literature suggests that nadir levels (i.e. lowest concentrations) of the glucocorticoid circadian profile are more likely to be altered by chronic stress than peak concentrations (Marti et al., 1993; Dhabhar & McEwen, 1997; Cure, 1989; Brennan et al., 2000). In humans, this would mean that basal differences in cortisol are more likely to be detected between groups of high and low chronic

stress when assessed in the afternoon rather than during the morning hours as in the experiment presented here. A recent study Powell et al. (2002) reported that evening cortisol measures are considerably more sensitive to background stress in women than morning concentrations. Indeed, several studies have reported increased afternoon cortisol concentrations while morning levels were not affected by chronic stress (Caplan et al., 1979; Melamed & Bruhis, 1996; Adam & Gunnar, 2001; Kobayashi et al., 1997; Opstadt 1994; Goejian et al., 1996; Yang et al., 2001). Some studies (Aardal-Erikson et al, 1999; Van Eck et al., 1996; Nicolson et al., 2000; Rose et al., 1982; Wüst et al., 2000; Sluiter et al., 2000; Sluiter et al., 1998; Fischer et al., 2000; Weitzman et al., 1994; Melamed et al., 1999) did not find any group differences in cortisol concentrations at all (neither nadir nor peak levels). Only one study has reported increased peak and unchanged nadir cortisol (Ockenfels et al., 1995). Thus, the lack of significant baseline differences in samples collected during the morning hours (i.e. the circadian peak in humans) seems to be in line with most of the animal and human literature.

However, the results of similar psychological self-report data and endocrine baseline levels in FR+ and FR- women presented here have to be critically discussed with respect to a recent report by Cohen and co-workers (2002a). This group investigated immune function, endocrine levels, and self-reported distress in 80 daughters of breast cancer patients (DBCP, two sample with n=39 and n=41, respectively) and 47 controls matched for age, education, and sociodemographic status. The two groups of DBCP women differed with respect to the cancer of their mother (primary localized breast cancer 1 year prior to the study (DBCP1) vs. recurrent breast cancer (localized or metastasized) 1 year prior to the study (DBCP2)).
They found significantly increased depressive symptoms (as measured with the BDI) and significantly elevated GSI scores (derived from the SCL-90-R, the parent instrument of the BSI used in our study). It should be noted that descriptively, the scores obtained from Cohen's sample (DBCP1 6.82$\pm$5.9; DBPC2 8.85$\pm$5.4; Controls 3.47$\pm$4.3; personal communication Miri Cohen) and the study presented here (FR+ 8.24$\pm$9.1; FR- 5.79$\pm$5.7) were very similar regarding the BDI. Thus, the significant findings in the Cohen study are most likely due to greater statistical power and / or the lower scores obtained in the control group. Even though the same factor may in part account for the different findings on the GSI, it also seems plausible that - above and beyond issues of statistical power - using the BSI instead of its longer version, the SCL-90-R, may have diminished the sensitivity of the instrument to detect subtle group differences. According to the theoretic model proposed by Rees et al. (2001), it

is also conceivable that stress effects may have been stronger in the Cohen sample, because they exclusively enrolled daughters of breast cancer patients with recent treatment. The experience of breast cancer in the family was thus very recent and potentially more imaginable, leading to increased availability of the experience. This has been found to be associated with increased risk perception and distress (see Introduction).

Cohen and colleagues also reported increased levels of stress hormones. The collection of the samples was conducted at a similar time of day (8:00h-9:00h), thus minimizing the circadian effects that may account for differences in comparison with our data. It should be noted that even with their higher statistical power, cortisol levels were only significantly elevated in women of the DBCP2 group, i.e. daughters of women with recurrent cancer. This suggests that the severity (both physiologically and psychologically) and recency (psychologically) of the relative's disease may play a role in basal glucocorticoid elevations. Cohen et al. (2002a) also reported significantly increased levels of norepinephrine and epinephrine in DBCP compared to controls as measured in urine. It should be noted that collection of first morning urine, as performed in their study, gives a measure of cumulative catecholamine secretion overnight whereas assaying norepinephrine and epinephrine from a single blood draw provides a "snapshot" picture of peripheral concentrations at the time of sampling. The results presented here thus refer to a different measure of catecholaminergic activity. Therefore, the findings are difficult to compare to the results provided by Cohen and coworkers. As suggested by rather moderate correlations of self-report and norepinephrine levels in their study (no significant associations with cortisol or epinephrine basal levels were found), factors other than psychological distress may account for observed differences. Further research is clearly needed in order to determine physiological as well as psychological predictors of endocrine baseline alterations in women at increased familial risk of breast cancer.

5.2. Alterations of endocrine stress responses in women at familial breast cancer risk

Although no increased self-reported levels of perceived stress, stress symptomatology, or depressive symptoms could be found in our sample, there were significant group differences in endocrine stress response patterns. In line with the study hypotheses, epinephrine and cortisol responses to acute psychological stress were significantly altered in women at increased risk for breast cancer. However, no alterations of stress response could be detected in norepinephrine levels.

For epinephrine, stress reactivity, as measured directly after stressor termination, was not affected by familial breast cancer risk: Both stressed groups, FR+ and FR-, evidenced significantly elevated epinephrine concentrations above control group levels. However, 15 minutes into the recovery phase (30 min post baseline), levels of FR- women had returned to control group levels while they were still significantly elevated in FR+ women.
For cortisol, the end product of HPA axis activation, reactivity directly post stressor as well as the first time point into the recovery period were significantly higher than control group levels in FR+ women. FR- women in the stress group did not differ significantly from the control group at any time.
The findings of delayed epinephrine recovery but unchanged reactivity are in line with a number of animal studies which showed endocrine recovery to be more sensitive than acute reactivity (Garcia et al., 2000; Marquez et al., 2002). It is also in accordance with data from human studies (see Linden et al., 1997). The pattern of the epinephrine response in FR+ women also resembles the "type III" allostatic load of McEwen's theory (1998), where a failure to shut off a response is hypothesized.

Given the fact that no differences in self-report distress could be found between the FR+ and FR- groups, it is tempting to speculate that the observed altered endocrine stress responses are a result of the biological (e.g. genetic) alterations likely to be found in the FR+ women rather than because of the psychological burden associated with an increased risk of breast cancer. However, we have to date no evidence that such a genetic link exists between hereditary breast cancer susceptibility and the genes encoding for stress responses (more detailed discussion of the genetic linkage see below). On the other hand, the observed endocrine alterations resemble a phenomenon extensively studied in animal chronic stress research. The finding of delayed recovery (epinephrine, cortisol) and increased reactivity (cortisol) are in line with a number of animal studies which have investigated acute reactivity to a novel (heterotypic) stressor on HPA and SNS stress responses after chronic stress exposure. A facilitated, i.e. stronger, endocrine stress response has compellingly been demonstrated for the hypothalamo-pituitary-adrenal (HPA) axis (Bhatnagar and Dallman, 1998, Weinstock et al., 1998, Bhatnagar et al., 1995, Young et al., 1990, Ottenweller et al., 1989, Johnson et al., 2002) and the sympathetic pathway (McCarty et al., 1988, Konarska et al., 1989, Weinstock et al., 1998). The stress model used in the human study presented here may best be described as a heterotypic stressor in a laboratory setting. Also, based on the literature, the psychological stress associated with increased familial risk of breast cancer is moderate rather

than severe. Therefore, our data is in good agreement with Antonio Armario's animal data showing that prior exposure to severe stressors leads to desensitization of the HPA response while moderate chronic stress is characterized by a facilitated response to a novel stressor (Andres et al., 1999).

Our results are also in line with the some of human studies on chronic stress and acute stress responses, though not all. Pike et al. (1997) reported an increased self-report, NK cell number and epinephrine stress reactivity in subjects with high life stress. In another study, Cacioppo et al. (2000) found that cortisol reactivity was increased while no changes were observed in catecholamine responses. Other studies enrolling teachers with high job strain (Benschop et al., 1994), unemployed participants (Ockenfels et al., 1995), high stress white collar workers (Van Eck et al., 1996), probationary firefighters (Roy et al., 1998) and volunteers with high self-reported stress (Matthews et al., 2001) did not find such effects. It is obvious that these types of chronic stress are considerably different in nature and may have different effects both psychologically as well as physiologically. As noted above, increased breast cancer risk and stress associated with caregiving may differ in certain characteristics. Some features may however be similar. It is tempting to speculate that unremitting stressors such as caring for a demented partner, or being at risk to develop a life-threatening disease may be more likely to show evidence of an impact on the acute reactivity of physiological stress systems than circumscribed stressors (see also Gump & Matthews, 1999). Consistent with this view, no alterations in endocrine stress reactivity to acute challenge were found in unemployed men and women (Ockenfels et al., 1994), or in male high school teachers with high job strain (Benschop et al., 1994). No human study has investigated the stressor characteristics which are responsible for alterations of acute physiological stress response alterations and little is known from the animal literature. This question remains to be addressed in future research.

Only one study has to date investigated physiological stress reactivity in women at increased breast cancer risk (Valdimarsdottir et al., 2002). In this sample, which in part comprised the same subjects as the study presented here, increased cardiovascular and immune (NK cell numbers) reactivity was seen. In the Valdimarsdottir study, no recovery patterns were examined. However, the direction of effects were comparable to the findings reported here for endocrine response alterations. Two other studies have investigated physiological alterations in women at familial breast cancer risk. Bovbjerg & Valdimarsdottir (1993) reported decreased basal NK cell activity. This was confirmed in a larger sample by Cohen et al. (2002a), who also reported increased emotional distress as well as cortisol and catecholamine concentrations. While Bovbjerg and Valdimarsdottir (1993) and Valdimarsdottir et al. (2002)

reported that physiological alterations were largely independent of self-report stress levels, Cohen et al. (2002a) were able to find moderate but statistically significant inverse correlations of self-report data and endocrine baseline parameters with immune parameters. Cohen et al. had a considerably larger FR+ sample (n=80), suggesting that while physiological alterations may be detected in smaller samples as well, the detection of increased self-report distress as well as the mediating effect of psychological data on endocrine, cardiovascular and immune alterations require larger samples. It is also conceivable that taking into account the women's experience with their relative's cancer (Rees et al., 2001) may help to identify subgroups of FR+ women with increased psychological distress and – potentially – even stronger alterations of endocrine stress responses.

5.3 Altered endocrine stress responses: Independent contribution of family history and depressive symptoms?

As shown in the results section, similar alterations as those seen between FR+ and FR- women, were seen when dividing the participants based on their score in the BDI. This was at least true for responses in epinephrine. Here, a delayed recovery was evident in women with higher scores (i.e. more depressive symptoms and higher scores of perceived stress). No changes were seen in reactivity. This is in line with the study hypotheses. It appears that of all self-report measures examined, BDI scores showed the strongest association with epinephrine stress response pattern. The significant stress response alterations in groups with higher or lower BDI scores were furthermore independent of scores on the PSS or GSI as well as VAS baseline levels.

As mentioned above, several studies have reported increased SNS response in chronically stressed animals (McCarty et al., 1988, Konarska et al., 1989, Weinstock et al., 1998). Chronic mild stress models are often used to mimic depression in animals, since they have been found to have good predictive validity, face validity, and construct validity (see Willner, 1997 for review). Transferring such models to humans is difficult. Furthermore, our sample had BDI scores in the non-clinical range and clinical depression was an exclusion criterion. It is important to note that a whole are of research investigates alterations in the endocrine system associated with clinical depression, especially the HPA axis (see Holsboer, 2001 for a brief overview). To review this literature is beyond the scope of this manuscript. It is further unclear whether mild and moderate depressive symptoms differ in degree or in kind when compared with major depression as a syndrome (see Flett et al., 1997, for a current analysis of

this "continuity" debate). I will therefore not discuss our findings with regard to endocrine alterations linked to clinical depression.

As described in the introduction, one human study has to date investigated physiological stress response to a speech task stressor in women with higher vs. lower BDI scores in the non-clinical range (Light et al., 1998). They report increased cardiovascular response (cardiac output) and no changes in epinephrine acute response while norepinephrine levels increased significantly stronger in women with high BDI scores compared to a group of women with lower BDI scores. We found no evidence to support the study hypothesis that women with higher BDI scores would have increased catecholamine reactivity to acute stress. The BDI-high and BDI-low groups showed comparable increases in epinephrine levels at the end of the stressor period, and neither showed any increase in norepinephrine levels. It should be noted that not all experimental stress studies find changes in the levels of both catecholamines, perhaps reflecting differential aspects of the specific stressors involved (Biondi & Picardi, 1999).

The lack of significant differences in epinephrine reactivity in the present study is consistent with previous findings by Light et al. (1998). In their study, the authors compared subgroups with higher or lower BDI. Recovery levels were not assessed. Women with higher BDI scores (BDI mean = 16.7) were found to have stronger norepinephrine reactivity than those of women with lower BDI scores (BDI mean = 2.2). Higher plasma levels were found in samples collected immediately following a 5-minute speech task. In addition to having somewhat more extreme BDI groups compared to the current study, other methodological details (e.g., types of stressors, stressor duration) may have contributed to the differences in outcomes between these two studies. One recently published study (Prüssner et al., 2003) reported that healthy subjects with higher levels of depressive symptoms (as measured with the Hamilton Depression Inventory) exhibited an increased cortisol response to awakening. This suggests that different types of acute "challenges" may detect a hyper-responsiveness of different neuroendocrine systems.

To our knowledge the present study is the first in the literature to provide evidence that higher baseline depressive symptom levels are predictive of slowed neuroendocrine recovery following a brief psychological stressor. We found that stress-induced epinephrine responses in healthy women with higher BDI scores took longer to return to basal levels. These results are consistent with recent theorizing on allostasis (McEwen, 1998). They are also in line with empirical findings in the animal literature suggesting that the recovery phase following termination of an acute stress is generally more sensitive to background distress than the acute

reactivity phase (Garcia et al., 2000; Marquez et al., 2002). Although both the theory and the animal literature would suggest that influences on recovery patterns following acute stress will not be limited to epinephrine responses, the absence of significant initial stress effects on norepinephrine (reactivity) in the present study precluded any investigation of the recovery phase. Additional research is thus needed to determine the impact of subclinical levels of depressive symptoms on the recovery phase of these and other stress-responsive systems.

Consistent with a possible mediating role, perceived stress (PSS) and general stress symptomatology (GSI) assessed for the week prior to the experimental session, as well as negative mood (VAS) assessed immediately prior to the session were significantly correlated with BDI scores. However, these differences did not account for the BDI group differences in the pattern of epinephrine response. While showing similar patterns of delayed recovery to that seen for the BDI groups, analyses based on PSS, GSI, and VAS scores (median split) did not reveal significant Time x Group interactions (p's ranged from .10 to .17). Moreover, the relationship between BDI scores and the pattern of epinephrine responses remained significant even after including the baseline distress variables as covariates in the analysis.

Although additional research is clearly necessary, the results of the present study are among the first in the literature to support an impact of subclinical levels of depressive symptoms on the pattern of responses to acute stress, independent of baseline levels of self-reported distress, or mood.

This may be of special interest since the importance of investigating subclinical depressive symptoms in their own right has been noted (Coyne & Gotlib, 1983). Accumulating evidence showing that health risks are associated not only with clinical depression but also with levels of depressive symptoms in the normal range (Frasure-Smith et al., 1995; Lesperance et al., 1996; Bush et al., 2001) has supported this view. Indeed, one recent large-scale study with the vast majority of subjects below clinical cutoffs on the Beck Depression Inventory (BDI), suggested a "dose-response" association between BDI scores and subsequent mortality following myocardial infarction (Lesperance et al., 2002). In this latter study, even subjects with BDI scores of 5 to 9 had significantly increased health risk compared to participants scoring below 5. The study presented here extends previous findings by Light and co-workers (1998) and provides preliminary cross-sectional evidence for an association of non-clinical depressive symptoms and delayed epinephrine recovery after acute psychological stress.

5.4. Alternative explanations: A common underlying biological susceptibility?

In the light of concerns raised by Dalton et al. (2002), one always has to consider the possibility that a common underlying biological susceptibility could determine the endocrine response to psychological stress as well as the risk for developing cancer. As described in the introduction, there is evidence that having a family history of breast cancer substantially increases a women's risk to develop the disease at one point in her life (Pharoah et al., 1997). Also, type specific genes have been detected that account for about 40% to 80% of the cases of hereditary breast cancer (see above). It is thus tempting to speculate that gene alterations associated with increased risk of breast cancer are also responsible for the observed alterations in endocrine stress responses. There is also evidence that endocrine stress responses, at least in the HPA axis, are in part genetically determined (Bartels et al., 2003). Kirschbaum et al. (1992) reported a genetic influence on the cortisol response to hCRH and, to a lesser extent, to psychosocial stress. In a study enrolling 52 monozygotic and 52 dizygotic twins, the same group observed a medium-sized genetic influence on the cortisol awakening response while no associations were found with circadian cortisol profiles (Wüst et al., 2000). Ruiz et al. (2001) reported that two novel mutations in the glucocorticoid receptor gene were found in 12 patients with primary cortisol resistance as defined by pathological dexamethasone suppression test. Preliminary reports have shown that certain polymorphisms of the glucocorticoid receptor gene are associated with increased reactivity to the Trier Social Stress Test (Wüst et al., 2002).

In a recent review, Mormede et al. (2002) conclude that a complex trait such as physiological stress responsiveness is not likely to be controlled by a single gene or a small group of genes. Rather, it seems to be regulated by a large number of different genes which interact and influence the behavioral and physiological output of the stressed individual. The two breast cancer susceptibility genes BRCA1 and BRCA2 are located on chromosome 13 and 17, respectively (Miki et al., 1994; Wooster et al., 1995). The gene encoding the human GR receptor is located on chromosome 5 (Theriault et al., 1989). To date, there is no evidence about a linkage between polymorphisms associated with HPA reactivity and genes identified as being relevant for breast cancer risk (BRCA1 and BRCA2).

From the study presented here, one cannot rule out a potential common genetic control of breast cancer risk and endocrine stress responses. The genotype of familial cancer risk or glucocorticoid receptor polymorphisms was not determined from the women participating in this experiment.

5.5. Limitations of the study

It is important to note that the study presented here has several limitations. Obviously, the sample of FR+ women was small. Therefore, analysis of subgroups which differ with respect to their breast cancer experience was not possible. There is considerable evidence from the literature as well as theoretical consideration that such differences may have a strong influence at least on psychological outcome. It is conceivable that there may be an interaction of FR+ and psychological distress on endocrine function. Since family history and depressive symptoms independently accounted for similar alterations at least in epinephrine responses, women with increased risk and increased levels of depressive symptoms may exhibit even further increased endocrine alterations. Future research should thus investigate possible group characteristics that may account for such an interaction (see below).

5.5.1. Modest changes: Comparison to the literature

The stress-induced changes in cortisol demonstrated here, while significant, are modest as has been the case in much of the previous literature with human experimental studies. The moderate changes in the present study may reflect the mild nature of the experimental stressors used. It should be emphasized that the magnitude of the changes reported here are very similar to results reported in the other experimental studies from the human literature conducted around the same time of day (i.e., morning). We report baseline levels around 13 • g/dl and an increase of 0.82 • g/dl in the FR+ stress group. The cited study by Cacioppo et al. (2000) reports mean baseline plasma cortisol levels of 10.4 • g/dl and 10.5 • g/dl, and mean change scores of 2.8 • g/dl and 0.91 • g/dl for caregivers and non-caregivers respectively. In another study, the same group (Cacioppo et al., 1995) reported baseline plasma levels of 12.2 • g/dl and 12.06 • g/dl midstressor. In a very recent study by Larson et al. (2001), mean cortisol plasma levels were 11.9 • g/dl at baseline and 11.2 • g/dl (original data reported in ng/ml) post stress after a brief speech task. All of these studies were carried out in the morning, i.e. time of peak basal cortisol levels, which are followed by rapid circadian declines. As discussed below, this time of day might be less than optimal for inducing large increases in cortisol. As can be seen from the examples cited above, the absence of a stress-induced increase in the FR- stress group is not unusual in healthy volunteers and further underlines the magnitude of the reactivity increase in the family history group in the present study. It should be noted that the biological and clinical impact of such modest changes has yet to be determined. However, as described in the introduction, recent theorizing regarding an increased allostatic load in individuals with a heightened physiological stress response (McEwen, 1998) suggest that modest differences in acute

reactivity to daily stressors may cumulatively contribute to long term adverse health consequences.

5.5.2. Timing of assessments and time of day

The sampling schedule around the stressor as well as the time of day the experiment is conducted can have profound effects on the results obtained. For example, catecholamines are part of the first wave of stress hormones released after onset of an acute stressor (Sapolsky et al., 2000) and concentrations are thought to peak within minutes. In the study presented here, there were no blood draws during the 15-minute stressor. It is therefore conceivable that catecholamine peak levels may not have been assessed. Thus, it cannot be excluded that alterations are in fact evident in epinephrine reactivity. However, it has been shown that epinephrine levels do not necessarily peak at this time and then start to decrease while the stressor is still present. Levels of epinephrine have been found to remain elevated throughout the course of protracted (e.g. two hours) exposure to experimental stressors (McCann et al., 1993).

Cortisol is thought to take considerably longer to reach peak concentrations (Saposky et al., 2000). Here, we found significant elevations of cortisol above control group levels in FR+ women only. As expected for assessments in the morning, a steep decline of cortisol concentrations is seen in the nonstressed control group. This is in line with the well-known circadian pattern of cortisol secretion. Since this study was conducted in the morning (8:00 AM – 10:00 AM), one would expect to see glucocorticoid concentrations decrease in participants who are not subjected to the stressor. It is also conceivable that a stronger cortisol stress response was masked by the underlying circadian decline in FR+ as well as FR- women. Dickerson & Kemeney (2002) have shown that studies conducted during the circadian nadir (i.e. afternoon in humans) are more likely to produce substantial cortisol responses. However, it should be noted that even under these less than optimal conditions to detect strong HPA activation, significant increases were seen in the FR+ stress group. Future research should look earlier in the stress response and do so during the afternoon, when circadian decline is less steep.

5.6. Implications for future research

In this study, altered endocrine responses (i.e. delayed recovery) have been found both for the HPA axis as well as the sympathetic pathway (epinephrine). Whereas differences in self-reported distress and depressive symptoms between FR+ and FR- women did not reach

statistical significance, the endocrine alterations were detected despite the limitations in statistical power. There are at least two possible explanations for this difference. It is conceivable that the time window assessed by the measures of psychological distress was too small. The questionnaires used here are designed to measure stress symptomatology (GSI) and perceived stress (PSS) in the past week. Depressive symptoms (BDI) were measured during the past month. It can therefore not be ruled out that measures covering a larger interval of time might have shown a closer association with endocrine stress responses.

It is however also possible that the endocrine stress system is more sensitive to effects of familial breast cancer risk than self-report data. Apart from providing additional physiological information on FR+, this finding hints to a potential pathway leading to an increased familial risk.

In the following sections, possible implications of delayed endocrine stress recovery and heightened reactivity in women at high risk for breast cancer are discussed and possible future research strategies are outlined. It is appreciated that some of these implications are highly speculative at this point in an area where only little empirical evidence is available. However, future research may benefit from the integration of different fields of research as outlined below.

Stress, cortisol, cognition, and health behaviors

Screening visits for breast cancer have been found to be highly stressful experiences (Aro et al., 2000), especially in women who are scheduled for a further mammogram because of inconclusive, unsatisfactory, or abnormal results (Sandin et al., 2002). Such visits may be particularly stressful for women who are aware of their increased cancer risk. Beyond the potential effects of even further increased cancer risk based on immune and metabolic alterations associated with stronger endocrine stress responses, the observed phenomenon may also have effects relevant to behavioral variables. It is well known that acute release of glucocorticoids (e.g. cortisol) inhibits cognitive processes (see Lupien & McEwen, 1997). Prolonged administration of glucocorticoids has also been found to inhibit cognitive function in healthy volunteers (Young et al., 1999). When given acutely, glucocorticoids significantly interfere with declarative and working memory in humans (e.g. Wolf et al., 2001).

Due to the increased cortisol response during such screening visits, FR+ women may potentially remember less information from the diagnostic interview and the screening guidelines provided by their physician. This could in turn affect compliance with surveillance suggestions and health behavior. According to the bio-behavioral model proposed by

Andersen et al. (1994), compliance and health behaviors are important factors influencing disease incidence as well as progression.

Endocrine responses in FR+ subgroups with strongly increased psychological distress.

As already mentioned above, with respect to the theoretical model proposed by Rees et al. (2001), women's personal experiences with a relative suffering from the disease may play a role in terms of their illness representations. It may be associated with increased psychological distress. It should thus further be studied if certain subgroups of FR+ women with a similar "objective" risk but different experiences differ on measures of perceived stress and endocrine stress responses. Zakowski et al. (1997) provided first evidence for a role of experience in psychological distress. Considering the human and animal endocrine literature, it appears likely that FR+ women with the experience of bereavement may exhibit even stronger endocrine acute stress response alterations. This warrants further investigation.

Additionally to the genetic breast cancer risk as established by family history assessment, these women may further be put at risk because of the alterations in acute endocrine stress responses. In this respect, it is of potential interest that similar epinephrine associations where found in women with higher scores on the BDI, even when statistically controlling for FR+. This suggest that for a subgroup of FR+ women who exhibit more depressive symptoms, detrimental effects of overly strong stress responses may even be stronger. To date, mostly cross-sectional data is available in support of McEwen's theory of allostatic load (McEwen & Seeman, 1999). Evidence from prospective studies to investigate the predictive value of endocrine stress response alterations as reported here is therefore clearly needed.

Genetic vs. environmental cause?

From the results of the study presented here, it is not possible to draw a conclusion about the underlying mechanism leading to increased endocrine responses and delayed recovery. As noted above, the familial disposition and the altered endocrine responses could be a result of the same or a related genetic disposition. Furthermore, in this study, we could not show a direct association of psychological distress and endocrine responses. The phenomenon may represent a genetic or a "acquired" hyper-activity of the HPA axis and the sympathetic pathway. It could also be the effect of a synergistic interaction of genetic preposition and stress. In any case, there is experimental evidence that HPA hyperactivity is progressive over time. That is, frequent activation of the HPA axis and chronically elevated glucocorticoid levels may (further) enhance hyperactivity of this system. Chronic stress has been found to

lead to glucocorticoid receptor downregulation, especially type I (MR) receptors in the hippocampus, resulting in a hyposuppressive state in rats (e.g. Young et al. 1990; Mizoguchi et al., 2001). It has further been demonstrated that chronic stress paradigms lead to downregulation of MR and GR mRNA expression (Francisca et al., 1996; Herman et al., 1995). Stress-induced glucocorticoid elevations have been linked to neuronal loss in the hippocampus due to the neurotoxic effects (Stein-Behrends et al., 1994). The hippocampus, where type I receptors (MR) are densely expressed, is considered to play a crucial role in the negative feedback loop of the HPA axis (DeKloet et al., 1998). It is tempting to speculate that heightened and longer-lasting glucocorticoid stress responses further increase HPA hyperactivity via hippocampal neurodegeneration. The causing factor as well as the development over time of endocrine response patterns remains to be elucidated in future studies.

Are endocrine stress response alterations involved in the psycho-biological interaction causing increased cancer incidence in FR+ women?

Above, the findings of this study have been discussed with respect to what the causing factor of the endocrine alterations may be (genetic vs. psychological/environmental). It has also been mentioned that, according to McEwen's theory, these alterations (through the effects and outcomes of the primary mediators) may lead to tertiary outcomes, including cancer (McEwen & Seeman, 1999, p. 40). It appears possible that the failure to shut-off acute stress responses is not merely an epiphenomenon of FR+ associated psychological distress ("acquired hyperactivity") but that it may actually represent one possible pathway linking familial breast cancer risk and breast cancer incidence.

What is known is that a family history of breast cancer is associated with increased likelihood for developing the disease (Pharoah et al., 1997). It also seems clear that while a number of gene mutations likely to determine genetic susceptibility have been identified, up to half of the hereditary breast cancers occur in the absence of such mutations. It has also been reported that mutation carriers are not necessarily affected by the disease (see section 2.1.1.). This suggests that carrying the gene mutation does not inevitably lead to disease and other factors may also play a role in determining whether or not a women will be affected by breast cancer. It is therefore tempting to speculate that some of the hereditary breast cancers are actually caused by a HPA hyperresponsivess, which may in part be genetically determined. As noted above, there is some evidence that such a genetic determination of endocrine stress responsiveness exists.

The study presented here provides the first preliminary evidence that FR+ women show altered endocrine stress responses closely resembling type III allostatic load as described by McEwen. Preliminary experimental evidence suggests that the HPA axis and the sympathetic pathway play a role in modulating physiological functions of suspected importance in tumor control. These include poorer repair of DNA and increases in the frequency of sister chromatid exchange, and NK cell function. Below, I will briefly highlight some examples. For instance, Shakkar and Ben-Eliyahu (1998) reported that in vivo administration of an adrenergic agonist (metaproterenol, MP) resulted in decreased NK cytotoxicity in a rat model. Furthermore, MP administration lead to a 10-fold increase in tumor cells metastases when a NK-sensitive tumor model was used. Adrenergic antagonists showed the opposite effects. DNA repair mechanisms and sister chromatid exchange have also been reported to be affected by stress in animal models (e.g. Glaser et al., 1985; Fischman & Kelly, 1987). The mechanism linking the physiological changes induced by stress and the observed changes in DNA repair and sister chromatid exchange is not known. Kiecolt-Glaser and Glaser (1999) suggest that "one or more stress hormones may mediate these responses" (p.1605), since the HPA axis and the autonomic nervous system are activated by stress.

It can be hypothesized that endocrine stress response alterations are one possible working pathway linking family history and breast cancer incidence. Future research will have to test whether such a hypothesis can be supported empirically. It would primarily be necessary to show the predictive value of endocrine alterations for cancer incidence independent of BRCA1 and BRCA2 mutations.

6. SUMMARY

Introduction: To date, little research has investigated the effects of chronic stressors on responsiveness of endocrine parameters and recovery after acute psychological stress in humans. Here, alterations in the endocrine responses to an acute stressor in women at familial risk of developing breast cancer is reported.

Methods: Familial breast cancer risk was assessed using a standard risk estimation model. Women were then subjected to a well-established, widely used laboratory stressor comprising a speech task and mental arithmetic. Endocrine response patterns of women with or without increased familial risk were compared to a randomly assigned control group.

Results: Stronger responses were seen in neuroendocrine activity of the major stress systems, the hypothalamo-pituitary-adrenal axis (plasma cortisol) and the sympathetic pathway (plasma epinephrine). For epinephrine, these alterations were evident through a delayed recovery pattern rather than increased reactivity. Cortisol showed both an increased reactivity as well as a delayed recovery in women at increased risk for breast cancer.

The two groups of women did not differ in the questionnaires used to assess perceived stress and depressive symptoms. However, alterations in epinephrine response similar to those when looking at familial risk of cancer as the grouping factor were seen in groups divided based on the scores on the BDI. Here, women with higher but non-clinical BDI scores exhibited similar reactivity but slower recovery of the epinephrine response. This effect was seen when statistically controlling for familial risk of breast cancer. It was further not mediated by higher scores on measures of perceived stress and stress symptomatology found in BDI-high women.

Implications: Results are in line with the theory of allostatic load. However, no mediating effect of self-reported distress on endocrine stress response alterations could be found. The observed enhanced reactivity and delayed recovery in cortisol and epinephrine stress response may indicate a potential pathway leading to detrimental health effects. Future research will have to determine if these response alterations are of predictive value. Such a delayed endocrine stress recovery may even be involved in the psycho-biological interactions leading to higher breast cancer incidence in this population.

References

Aardal-Eriksson, E., Eriksson, T.E., Holm, A.C., Lundin, T. (1999). Salivary cortisol and serum prolactin in relation to stress rating scales in a group of rescue workers. Biol. Psychiatry 46, 850-5.

Adam, E.K., Gunnar, M.R. (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. Psychoneuroendocrinol. 26,189-208.

Ader, R., Cohen, N. (1975). Behaviorally conditioned immunosuppression. Psychsom. Med. 37, 333-340.

Ader, R., Cohen, N., Bovbjerg, D. (1982). Conditioned suppression of humoral immunity in the rat. J. Comp. Physiol. Psychol. 96, 517-521.

Ader, R. (2000). On the development of psychoneuroimmunology. Eur. J. Pharmacol. 405, 167-176.

Ader, R (Ed), (2001). Psychoneuroimmunology. San Diego: Academic Press.

Adler, A. (1943). Neuropsychiatric complications in victims of Boston's Cocoanut Grove disaster. JAMA 17, 1098-1101.

Ahearn, E.P. (1997). The use of visual analog scales in mood disorders: a critical review. J. Psychiatry Res., 31, 569-579.

Al'Absi, M., Bongard, S., Buchanan, T., Pincomb, G.A., Licinio, J., Lovallo, W.R. (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. Psychophysiology 34, 266-275.

Al' Absi, M, Lovallo, WR, McKey, B, Sung, BH, Whitsett, TL, Wilson, MF. (1998). Hypothalamic-pituitary-adrenocortical responses to psychological stress and caffeine in men at high and low risk for hypertension. Psychosom. Med. 60, 521-527.

American Cancer Society (2003). Cancer facts and figures: 2003. American Cancer Society, New York. Available online at http://www.cancer.org

Andersen, B., Kiecolt-Glaser, J.K., Glaser, R. (1994). A biobehavioral model of cancer stress and disease course. Am. Psychologist 49, 389-404.

Andres, R., Marti, O., Armario, A. (1999). Direct evidence of acute stress-induced facilitation of ACTH response to subsequent stress in rats. Am. J. Physiol. 277, R863-R868.

Arbeitsgemeinschaft Bevölkerungsbezogener Krebsregister in Deutschland (2002). Krebs in Deutschland: Häufigkeiten und Trends.Saarbrücken: Braun.

Aro, A.R., Pilvikki-Absetz, S., van Elderen, T.M., van der Ploeg, E., van der Kamp, L.J. (2000). False-positive findings of mammography screening induces short-term distress – breast cancer specific concerns prevail longer. Eur. J. Cancer 36, 1089-1097.

Arver, B., Du, Q., Chen, J., Luo, L., Lindblom, A. (2000). Hereditary breast cancer: A review. Cancer Biol. 10, 271-288.

Baider, L., Ever-Hadani, P., Kaplan De-Nour, A. (1999). Psychological distress in healthy women with familial breast cancer: Like mother, like daughter? Int J Psychiatry Med 29, 411-420.

Baron, R.M., Kenny, D.A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J. Pers. Soc. Psychol. 51(6), 1173-82.

Bartels, M., Van den Berg, M., Sluyter, F., Boomsma, D.I., de Geus, E.J.C. (2003). Heritability of cortisol levels: Review and simultaneous analysis of twin studies. Psychoneuroendocrinol. 28, 121-137.

Baum, A., Krantz, D.S., Gatchel, R.J. (1997). An introduction to health psychology. Boston: McGraw-Hill.

Barnes, R.F., Raskind, M., Gumbrecht, G., Halter, J.B. (1982). The effects of age on the plasma catecholamine response to mental stress in man. J. Clin. Endocrinol. Metab. 54, 64-69.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J. (1961). An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561-571.

Beck, A.I., Steer, R.A., Garbin, M.G. (1988). Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clin. Psychol. Rev. 27, 77-100.

Benschop, R.J., Brosschot, J.F., Godaert, G.L., De Smet, M.B., Geenen, R., Olff, M., Heijnen, C.J., Ballieux. R.E. (1994). Chronic stress affects immunologic but not cardiovascular responsiveness to acute psychological stress in humans. Am. J. Physiology 266, R75-R80.

Besedovsky, H.O., Sorkin, E. (1977). Network of immune-endocrine interactions. Clin. Exp. Immunol. 27, 1-12.

Besedovsky, H.O., Del Rey, A. (1996). Immune-neuro-endocrine interactions: Facts and hypotheses. Endocr Rev 17, 64-102.

Biondi, M., Picardi, A. (1999). Psychological stress and neuroendocrine function in humans: The last two decades of research. Psychother Psychosom 68, 114-150.

Bhatnagar, S., Mitchell, J.B., Betito, K., Boksa, P., Meaney, M.J. (1995). Effects of chronic intermittent cold stress on pituitary adrenocortical and sympathetic adrenomedullar function. Physiol. Behav. 57(4), 633-639.

Bhatnagar, S., Dallman, M. (1998). Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. Neuroscience 84, 1025-1039.

Bovbjerg, D.H., Valdimarsdottir, H.B. (1993). Familial cancer, emotional distress, and low natural cytotoxic activity in healthy women. Ann Oncol 4, 745-752.

Bovbjerg, D.H., Valdimarsdottir, H.B., Zachariae, R. (1999). Psychoneuroimmunology in oncology. In : Schedlowski, M., Tewes, U. Psychoneuroimmunology: An interdisciplinary introduction. New York: Kluwer Academic.

Bovbjerg, D.H., Valdimarsdottir, H.B. (2001). Interventions for healthy individuals at familial risk for cancer. In: Baum, A. and Anderson, B.L. (Ed.), Psychosocial interventions for cancer. American Psychological Association, Washington, pp. 305-320.

Brain, K., Norman, P., Gray, J., Mansel, R. (1999). Anxiety and adherence to breast self-examination in women with a family history of breast cancer. Psychosom Med 61, 181-187.

Brennan, F.X., Ottenweller, J.E., Seifu, Y., Zhu, G., Servatius, R.J. (2000). Persistent stress-induced elevations of urinary corticosterone in rats. Physiol. Behav. 71, 441-446.

Bush, D.E., Ziegelstein, R.C., Tayback, M., Richter, D., Stevens, S., Zahalsky, H., Fauerbach, J.A. (2001). Even minimal symptoms of depression increase mortality risk after acute myocardial infarction. Am J Cardiol 88(4), 337-41.

Butow, P.N., Hiller, J.E., Price, M.A., Thackway, S.V., Kricker, A., Tennant, C.C. (2000). Epidemiological evidence for a relationship between life events, coping style, and personality factors in the development of breast cancer. J. Psychosom. Res. 49, 169-181.

Cannon, W.B. (1929). Organization of physiological homeostasis. Physiol Rev 9, 399-431.

Cacioppo, J.T., Malarkey, W.B., Kiecolt-Glaser, J.K., Uchino, B.N., Sgoutas-Emch, S.A., Sheridan, J.F., Berntson, G.G., Glaser R. (1995). Heterogeneity in neuroendocrine and immune responses to brief psychological stressors as a function of autonomic cardiac activation. Psychosom. Med. 57(2),154-64.

Cacioppo, J.T., Burleson, M.H., Poehlmann, K.M., Malarkey, W.B., Kiecolt-Glaser, J.K., Berntson, G.G., Uchino, B.N., Glaser, R. (2000). Autonomic and neuroendocrine responses to mild psychological stressors: effects of chronic stress on older women. Ann. Behav. Med. 22, 140-148.

Caplan, R.D., Cobb, S., French, J.R. Jr. (1979). White collar work load and cortisol: disruption of a circadian rhythm by job stress? J. Psychosom. Res. 23, 181-92.

Carbone, J.E., Ohm, D.P. (2002). Immune dysfunction in cancer patients. Oncology 16, 11-18.

Chamberlain, J., Palli, D. (1993). Evidence of the effectiveness of breast cancer screening. In: Gad, A., Rossellini del Turco, M. (Eds.). Breast cancer screening in Europe. Berlin: Springer Verlag, pp. 15-23.

Champion, V. (1987). The relationship of breast self examination to health belief model variables. Res. Nurs. Health 10, 375-382.

Chung, K.K.K., Martinez, M., Herbert, J. (2000). C-fos expression, behavioral, endocrine, and autonomic responses to acute social stress in male rats after chronic restraint: modulation by serotonin. Neuroscience 95, 453-463.

Claus, E.B., Risch, N., Thompson, W.D. (1990). Age at onset as an indicator of familial risk of breast cancer. Am. J. Epidemiol. 131, 961-972.

Claus, E.B., Risch, N., Thompson, W.D. (1991). Genetic analysis of breast cancer in the cancer and steroid hormone study. Am. J. Hum. Genet. 48, 232-242.

Claus, E.B., Risch, N., Thompson, W.D. (1994). Autosomal dominant inheritance of early-onset breast cancer. Cancer 73, 643-651.

Claus, E.B., Schildkraut, J.M., Thompson, W.D., Risch, N.J. (1996). The genetic attributable risk of breast cancer and ovarian cancer. Cancer 77, 2318-2324.

Cohen, M., Klein, E., Kuten, A., Fried, G., Zinder, O., Pollack, S. (2002a). Increased emotional distress in daughters of breast cancer patients is associated with decreased natural killer cytotoxic activity, elevated levels of stress hormones and decreased secretion of TH1 cytokines. Int. J. Cancer 100, 347-354.

Cohen, S., Kamarck, T., Mermelstein, R. (1983). A global measure of perceived stress. J. Health Soc. Behav. 24(4), 385-96.

Cohen, S., Hamrick, N., Rodriguez, M.S., Feldman, P.J., Rabin, B.S., Manuck, S.B. (2002b). Reactivity and vulnerability to stress-associated risk for upper respiratory illness. Psychosom. Med. 64, 302-310.

Corder-Bolz, C.R. (1978). The evaluation of change: New evidence. Educ. Psychol. Measure. 38, 959-976.

Cottingham, J., Stoothoff, R., Murdoch, D., Kenny, A. (1988). Descartes: Selected philosophical writings. Cambridge: University Press.

Couch, F.J., DeShano, M.L., Blackwood, M.A., Calzone, K., Stopfer, J., Campeau, L., Ganguly, A., Rebbeck, T., Weber, B.L. (1997). BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. New Engl. J. Med. 336, 1409-1415.

Coyne, J.C., Gotlib, I.H. (1983). The role of cognition in depression: a critical appraisal. Psychol Bull 94(3), 472-505.

Cure, M. (1989). Plasma corticosterone response in continuous versus discontinuous chronic heat exposure in rat. Physiol. Behav. 45, 1117-22.

Dalton, S.O., Boesen, E.H., Ross, L., Schapiro, C. (2002). Mind and cancer: Do psychological factors cause cancer. Eur. J. Cancer 38, 1313-1323.

Davidson, L., Vandogen, R., Rouse, I.L., Beilin, I.J., Tunney, A. (1984). Sex-related differences in resting and stimulated plasma noradrenaline and adrenaline. Clin. Sci. 67, 347-352.

Decruyenaere, M., Evers-Kiebooms, G., Welkenhuysen, M., Denayer, L., Claes, E. (2000). Cognitive representations of breast cancer, emotional distress and preventive health behaviour: A theoretical perspective. Psycho-Oncol. 9, 528-536.

De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M. (1998). Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19: 269-301.

Del Rio, G., Velardo, A., Zizzo, G., Avogaro, A., Cipolli, C., Della Casa, L., Marrama, P., MacDonald, I.A. (1994). Effects of estradiol on the sympathoadrenal response to mental stress in normal men. J. Clin. Endocrinol. Metab. 79, 836-840.

Derogatis, L.R., Rickels, K., Rock, A.F. (1976). The SCL-90-R and the MMPI: A step in the validation of a new self-report scale. Br. J. Psychiatry 128, 280-289.

Derogatis, LR, Melisaratos, N. (1983). The Brief Symptom Inventory: an introductory report. Psychol. Medicine 13(3), 595-605

Derogatis, L.R., Spencer, P. (1982). The brief symptom inventory (BSI) administration scoring and procedures manual-I. Baltimore: copyrighted manuscript.

Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Korner, A., Schmider, J., Standhardt, H., Lammers, C.H., Heuser, I. (1997). With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. Life Sci. 61, 2239-2246.

Dhabhar, F.S., McEwen, B.S. (1997). Acute stress enhances while chronic stress suppresses cell-mediated immunity *in vivo*: A potential role for leukocyte trafficking. Brain Behav. Immun. 11, 286-306.

Dickerson, S.S., Kemeney, M.E. (2002). Predictors of cortisol reactivity to acute psychological stress: A meta-analytic review. [Abstract] Psychosom Med 64, 105. Presented at the Annual Meeting of the American Psychosomatic Society, Barcelona, Spain, March 2002.

Dimsdale, J.F. (1984). Generalizing from laboratory studies to field studies of human stress physiology. Psychosom. Med. 46, 463-469.

Drossaert, C.C.H., Boer, H., Seysel, E.R. (1996). Perceived risk, anxiety, mammography uptake, and breast self examination of women with family history of breast cancer: The role of knowing to be at increased risk. Cancer Detect. Prevent. 20, 76-85.

Dunkin, J., Anderson-Hanley, C. (1998). Dementia caregiving burden: A review of the literature and guidelines for assessment and intervention. Neurology 51 (1 Suppl 1), S53-S60.

Earle, T.L., Linden, W., Weinberg, J. (1999). Differential effects of harassment on cardiovascular and salivary cortisol stress reactivity in women and men. J. Psychosom. Res. 46(2), 125-141.

Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S. (2000). The sympathetic nerve – an integrative interface between two supersystems: The brain and the immune system. Pharmacol. Rev. 52, 595-638.

Epel, E.S., McEwen, B.S., Seeman, T., Matthews, K., Castelazzo, G., Brownell, K.D., Bell, J., Ickovics, J.R. (2000). Stress and body shape: Stress-induced cortisol secretion is consistently greater among women with central fat. Psychosom. Med. 62, 623-632.

Epstein, S.A., Lin, T.H., Audrain, J., Stefanek, M., Rimer, B., Lerman, C., and the Risk Breast Cancer Consortium (1997). Excessive breast self examination among first degree relatives of newly diagnosed breast cancer patients. Psychosomatics 38, 253-261.

Erblich, J., Bovbjerg, D.H., Valdimarsdottir, H.B. (2000a). Looking forward and back: distress among women at familial risk for breast cancer. Ann Behav Med 22, 53-59.

Erblich, J., Bovbjerg, D.H., Valdimarsdottir, H.B. (2000b). Psychological distress, health beliefs, and frequency of breast self-examination. J Behav Med 23, 277-292.

Eriksen, H.R., Olff, M., Murison, R., Ursin, H. (1999). The time dimension in stress responses: Relevance for survival and health. Psychiatry Res. 85, 39-50.

Euhus, D.M., Smith, K.C., Robinson, L., Stucky, A., Olopade, O.I., Cummings, S., Garber, J.E., Chittenden, A., Mills, G.B., Rieger, P., Esserman, L., Crawford, B., Hughes, K.S., Roche, C.A., Ganz, P.A., Seldon, J., Fabian, C.J., Klemp, J., Tomlinson, G. (2002). Pretest prediction of BRCA1 or BRCA2 mutation by risk counselor and the computer model BRCAPRO. J. Natl. Cancer Inst. 94, 844-851.

Evans, E. (1926). A psychological study of cancer. New York: Dodd, Mead.

Fischer, J.E., Calame, A., Dettling, A.C., Zeier, H., Fanconi, S. (2000). Objectifying psychomental stress in the workplace--an example. Int Arch Occup Environ Health 73 (Suppl), S46-52.

Fischman, H.K., Kelly, D.D. (1987). Sister chromatid exchanges induced by behavioral stress. Ann. N.Y. Acad. Sci. 496, 426-435.

Flett, G.L., Vredenburg, K., Krames, L. (1997). The continuity of depression in clinical and nonclinical samples. Psychol. Bulletin 121, 395-416.

Francisca, G., Abdeljalil, L., de Kloet, E.R., Armario, A.(1996). Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at adreno-cortical level. Neuroendocrinology 63, 327-337.

Frasure-Smith, N., Lesperance, F., Talajic, M. (1995). Depression and 18-month prognosis after myocardial infarction. Circulation 91(4), 999-1005.

Gail, M.H., Brinton, L.A., Byar, D.P., Corle, D.K., Green, S.B., Schair, C. (1989). Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J. Natl. Cancer Inst. 81, 1879-1886.

Garber, J.E., Henderson, I.C., Love, S.M., Gelman, R. (1991). Management of high-risk groups. In: Harris, J.R., Hellman, S., Henderson, I.C., Kinne, D.W. (Eds). Breast disease. New York: Lippicott Company, pp. 153-165.

Garcia, A., Marti, O., Valles, A., Dal-Zotto, S., Armario, A. (2000). Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. Neuroendocrinol. 72(2), 114-25.

Gerra, G., Zaimovic, A., Mascetti, G.G., Gardini, S., Zambelli, U., Timpano, M., Raggi, M.A., Brambilla, F. (2001). Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. Psychoneuroendocrinol. 26, 91-107.

Gilbert, D.G., Dibb, W.D., Plath, L.C., Hiyane, S.G. (2000). Effects of nicotine and caffeine, seperately and in combination, on EEG topography, mood, heart rate, cortisol, and vigilance. Psychophysiology 37, 583-595.

Gilbrar, O. (1998). Coping with threat. Implications for women with a family history of breast cancer. Psychosomatics 39, 329-339.

Glaser, R., Thom, B.E., Tarr, K.L., Kiecolt-Glaser, J.K., D'Ambrosio, S.M. (1985). Effects of stress on methyltransferase synthesis. An important DNA repair enzyme. Health Psychol 4, 403-412.

Goenjian, A.K., Yehuda, R., Pynoos, R.S., Steinberg, A.M., Tashjian, M., Yang, R.K., Najarian, L.M., Fairbanks, L.A. (1996). Basal cortisol, dexamethasone suppression of cortisol, and MHPG in adolescents after the 1988 earthquake in Armenia. Am. J. Psychiatry 153, 929-34.

Greer, S. (1983). Cancer and the mind. Br. J. Psychiatry 143, 535-543.

Gump, B.B., Matthews, K.A. (1998). Vigilance and cardiovascular reactivity to subsequent stressors in men: A preliminary study. Health Psychol 17, 93-96.

Gump, B.B., Matthews, K.A. (1999). Do chronic stressors influence reactivity to and recovery from acute stressors? J Appl Soc Psychol 29, 469-494.

Haddad, J.J., Saade, N.E., Safieh-Garabedian, B. (2002). Cytokines and neuro-immune-endocrine interactions: A role for the hypothalamic-pituitary-adrenal revolving axis. J. Neuroimmunol. 133, 1-19.

Haddan, J.W., Haddan, E.M., Middleton, E. (1970). Lymphocyte blast transformation: I. Demonstration of adrenergic receptors in human peripheral lymphocytes. J. Cell. Immunol. 1, 583-595.

Hadjiconstatinou, M., McGuire, L., Duchemin, A.M., Laskowski, B., Kiecolt-Glaser, J., Glaser, R. (2001). Changes in plasma nerve growth factor levels in older adults associated with chronic stress. J Neuroimmunol 116, 102-106.

Helzlsouer, K.J. (1993). Epidemiology, early detection, and prevention of breast cancer. Curr. Opin. Oncol. 5, 955-959.

Helzlsouer, K.J., Ford, D.E., Hayward, S.A., Midzenski, M., Perry, H. (1994). Perceived risk of cancer and practice of cancer prevention behaviours among employees in an oncology center. Prevent. Med. 23, 302-308.

Hennig, J., Friebe, J., Ryl, I., Kramer, B., Bottcher, J., Netter, P. (2000). Upright posture influences salivary cortisol. Psychoneuroendocrinol. 25, 69-83.

Herman, J.P., Adams, D., Prewitt, C. (1995). Regulatory changes in neuroendocrine stress-integrative circuit produced by variable stress paradigm. Neuroendocrinol. 61, 180-190.

Holsboer, F. (2001). Stress, hypercortisolism, and corticosteroid receptors in depression: Implications for therapy. J. Affect. Disord. 62, 77-91.

Imai, K., Matsuyama, S., Miyake, S., Suga, K., Nakatchi, K. (2000). Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356, 1795-1799.

Johnson, J.D., O'Connor, K.A., Deak, T., Spencer, R.L., Watkins, L.R., Maier, S.F. (2002). Prior stressor exposure primes the HPA axis. Psychoneuroendocrinol. 27, 353-365.

Jorgensen, L.S., Christiansen, P., Raundahl, U., Ostgaard, S., Christensen, N.J., Fenger, M., Flachs, H. (1990). Autonomic response to an experimental psychological stressor in healthy subjects: Measurement of sympathetic, parasympathetic, and pituitary-adrenal parameters. Scand. J. Clin. Lab. Invest. 50, 82-829.

Kamarck, T.W. (1982). Recent developments in the study of cardiovascular reactivity: Contributions from psychometric theory and social psychology. Psychophysiol. 29, 491-503.

Kaplan, J.R., Petterson, K., Manuck, S.B., Olsson, G. (1991). Role of sympatho-adrenal medullary activation in the initiation and progression of atheriosclerosis. Circulation 84 (Suppl VI), VI-23-VI-32.

Kash, K.M., Holland, J.C., Halper, M.S., Miller, D.G. (1992). Psychological distress and surveillance behaviors of women with a family history of breast cancer. J Natl Cancer Inst 84, 24-30.

Kemmer, F.W., Bisping, R., Steingrüber, H.J., Baar, H., Hardtmann, F., Schlaghecke, R., Berger, M. (1986). Psychological stress and metabolic control in patients with type I diabetes mellitus. N. Engl. J. Med. 314, 1087-1084.

Kiecolt-Glaser, J.K., Dura, J.R., Speicher, C.E., Trask, O.J., Glaser, R. (1991). Spousal caregivers of demetia victims: Longitudinal changes in immunity and health. Psychosom Med 53, 345-362.

Kiecolt-Glaser, J.K., Marucha, P.T., Malarkey, W.B., Mercado, A.M., Glaser, R. (1995). Slowing of wound healing by psychological stress. Lancet 346, 1194-1196.

Kiecolt-Glaser, J.K., Glaser, R. (1999). Psychoneuroimmunology and cancer: Fact or fiction? Eur. J. Cancer 35, 1603-1607.

Kirschbaum, C., Wüst, S., Faig, H.G., Hellhammer, D.H. (1992a). Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in humans. J Clin Endocrin Metab 75, 1526-1530.

Kirschbaum, C., Wüst, S., Hellhammer, D. (1992b). Consistent sex differences in cortisol responses to psychological stress. Psychosom. Med. 54, 648-657.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.K. (1993). The "Trier Social Stress Test" – a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76-81.

Kirschbaum, C., Hellhammer, D.H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. Psychoneuroendocrinol.19, 313-333.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H. (1999). Impact of gender, menstrual cycle phase and oral contraceptive use on the activity of the hypothalamo-pituitary-adrenal axis. Psychosom. Med. 61, 154-162.

Kobayashi, F., Furui, H., Akamatsu, Y., Watanabe, T., Horibe, H. (1997). Changes in psychophysiological functions during night shift in nurses. Influence of changing from a full-day to a half-day work shift before night duty. Int. Arch. Occup. Environ. Health 69(2), 83-90.

Kudielka, B.M., Schmid-Reinwald, A.K., Hellhammer, D.H., Kirschbaum, C. (1999). Psychological and endocrine responses to psychosocial stress and dexamethasone/corticotropin-releasing hormone in healthy postmenopausal women and young controls: The impact of age and a two-week estradiol treatment. Neuroendocrinol 70, 422-430.

Kudielka, B.M., Schmid-Reinwald, A.K., Hellhammer, Schurmeyer, T., D.H., Kirschbaum, C. (2000). Psychosocial stress and HPA functioning: No evidence for a reduced resilience in healthy elderly men. Stress 3, 229-240.

Konorska, M., Stewart, R.E., McCarty, R. (1989). Sensitization of sympathetic-adrenal medullary responses to a novel stressor in chronically stressed laboratory rats. Physiol. Behav. 46, 129-135.

Larson, M.R., Ader, R., Moynihan, J.A. (2001). Heart rate, neuroendocrine, and immunological reactivity in response to an acute laboratory stressor. Psychosom. Med. 63, 493-501.

Lazarus, R.S., Folkman, S. (1984). Stress, appraisal, and coping. New York: Springer.

LeBlanc, J., Cote, J., Jobin, M., Labrie, A. (1979). Plasma catecholamines and cardiovascular responses to cold and mental activity. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 47, 1207-1211.

Leventhal, H., Benyamini, N. (1997). Lay beliefs about health and illness. In: Baum, A., Newman, S., Weinman, J., West, R., McNanus, C. (Eds). Cambridge handbook of psychology, health and medicine. Cambridge: Cambridge University Press.

Levine, S., Ursin, H. (1991). What is stress? In: Brown, M.R., Koob, G.F., Rivier, C. (Eds). Stress – neurobiology and neuroendocrinology. New York: Dekker, pp 3-21.

Lerman, C., Daly, M., Sands, C., Balshem, A., Lustbader, E., Heggan, T., Goldstein, L., James, J., Engstrom, P. (1993). Mammography adherence and psychological distress among women at breast cancer risk. J. Natl. Cancer Inst. 85, 1974-1080.

Lerman, C., Schwarz, M. (1993). Adherence and psychological adjustment among women at high risk for breast cancer. Breast Cancer Res Treat 8, 145-155.

Lerman, C., Kash, K., Stefanek, M. (1994). Younger women at increased risk for breast cancer: Perceived risk, psychological well-being, and surveillance behavior. Monogr Natl Cancer Inst 16, 171-176.

Lerman, C., Schwartz, M.D., Lin, T.H., Hughes, C., Narod, S., Lynch, H.T. (1997). The influence of psychological distress on use of genetic testing for cancer risk. J. Consult. Clin. Psychol. 65, 414-420.

Lespérance, F., Frasure-Smith, N., Talajic, M. (1996). Major depression before and after myocardial infarction: its nature and consequences. Psychosom Med 58(2), 99-110.

Leproult, R., Copinschi, G., Buxton, O., Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening. Sleep 20, 865-70.

Light, K.C., Kothandapani, R.V., Allen, M.T. (1998). Enhanced cardiovascular and catecholamine responses in women with depressive symptoms. Int J. Psychophysiol 28(2): 157-66.

Lindberg & Wellisch (2001). Axienty and compliance among women at high risk for breast cancer. Ann. Behav. Med. 23(4), 298-303.

Linden, W., Earle, T.L., Gerin, W., Christenfeld, N. (1997). Physiological stress reactivity and recovery: Conceptual siblings separated at birth? J. Psychosom. Res. 42, 117-135.

Lovallo, W.R., Al' Absi, M., Blick, K., Whitsett, T.L., Wilson, M.F. (1996). Stress-like adrenocorticotropin responses to caffeine in young healthy men. Pharmacol. Biochem. Behav. 55, 365-9.

Lupien, S.J., McEwen, B.S. (1997). The acute effects of corticosteroids on cognition: Intregration of animal and human model studies. Brain Res. Rev. 24, 1-27.

Lupien, S.J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N.P., Thakur, M., McEwen, B.S., Hauger, R.L., Meaney, M.J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69-73.

Marquez, C., Belda, X., Armario, A. (2002). Post-stress recovery of pituitary-adrenal hormones and glucose, but not the response during exposure to the stressor, is a marker of stress intensity in highly stressful situations. Brain Res. 926(1-2), 181-5.

Marti, O., Gavalda, A., Jolin, T., Armario, A. (1993). Effect of regularity of exposure to chronic immobilization stress on the circadian pattern of pituitary adrenal hormones, growth hormone, and thyroid stimulating hormone in the adult male rat. Psychoneuroendocrinol. 18, 67-77.

Mason, J.W. (1971). A re-evaluation of the concept of “non-specificity” in stress theory. J. Psych. Res. 8, 323-333.

Matthews, K.A., Gump, B.B., Owens, J.F. (2001). Chronic stress influences cardiovascular and neuroendocrine responses during acute stress and recovery, especially in men. Health Psychol 20, 403-410.

McCann, B.S., Carter, J., Vaughan, M., Raskind, M., Wilkinson, C.W., Veith, R.C. (1993). Cardiovascular and neuroendocrine responses to extended laboratory challenge. Psychosom. Med. 55, 497-504.

McCaul, K.D., Branstetter, A.D., Schroeder, D.M., Glasgow, R.E. (1996). What is the relationship between breast cancer risk and mammography screening? A meta-analytic review. Health Psychol 6, 423-429.

McCormick, C.M., Kehoe, P., Kovacs, S. (1998). Corticosterone release in response to repeated, short episodes of neonatal isolation: Evidence of sensitization. Int J Dev Neurosci 16, 175-185.

McEwen, B.S., Biron, C.A., Brunson, K., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L., Weiss, J.M. (1997). Neural-endocrine-immune interactions: The role of adrenocorticoids as modulators of immune function in health and disease. Brain Res. Rev. 23, 79-133.

McEwen, B.S., (1998). Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171-179.

McEwen, B.S., Seeman, T. (1999). Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann. N.Y. Acad. Sci. 896, 30-47.

McEwen, B.S. (2000). The neurobiology of stress: From serendipity to clinical relevance. Brain Res. 886, 172-189.

McEwen, B.S. (2001). Plasticity of the hippocampus: Adaptation to chronic stress and allostatic load. Ann. N.Y. Acad. Sci. 933, 265-277.

Melamed, S., Bruhis, S (1996). The effects of chronic industrial noise exposure on urinary cortisol, fatigue and irritability: a controlled field experiment. J. Occup. Environ. Med. 38, 252-6.

Melamed, S., Ugarten, U., Shirom, A., Kahana, L., Lerman, Y., Froom, P. (1999). Chronic burnout, somatic arousal and elevated salivary cortisol levels. J. Psychosom. Res. 46, 591-8.

Miki, Y., Swensen, J., Shattuck-Eides, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W. (1994). A strong candidate for breast and ovarian cancer susceptibility gene BRCA1. Science 266, 66-71.

Miller, F.R., Jones, H.W. (1948). The possibility of precipitating the leukemia state by emotional factors. Blood 8, 880-885.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D.H., Tabira, T. (2001). Chronic stress differentially regulates glucocorticoid negative feedback response in rats. Psychoneuroendorinol. 26, 443-59.

Modell, E., Yokoyama, Y., Irahara, M., Aono, T. (1994). Endocrine and behavioral responses to psychological stress in hyperandrogenic women. Fertil Steril 53, 454-459.

Mormede, P., Courvoisier, H., Ramos, A. Marissal, A.N., Ousova, O., Desautes, C., Duclos, M., Chaouloff, F., Moisan, M.P. (2002). Molecular genetic approaches to investigate individual variations in behavioral and neuroendocrine responses. Psychoneuroendocrinol. 27, 563-583.

Munck, A., Guyre, P.M., Holbrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25-44.

Neumann, P.J., Polister, P.E. (1992). Risk and optimality. In: Yates, J.F. (Ed). Risk-taking behavior. New York: Wiley, pp. 27-46.

Nicolson, N.A., van Diest, R.(2000). Salivary cortisol patterns in vital exhaustion. J. Psychosom. Res. 49(5), 335-342.

Ockenfels, M.C., Porter, L. Smyth, J., Kirschbaum, C., Hellhammer, D.H., Stone, A.A. (1995). Effects of chronic stress associated with unemployment on salivary cortisol: Overall cortisol levels, diural rhythm, and acute stress reactivity. Psychosom Med 57, 460-467.

Opstad, K. (1994). Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. Eur. J. Endocrinol. 131, 56-66.

Pacak, K., Palkovits, M. (2001). Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. Endocr. Rev. 22, 502-548.

Parkin, D.M., Pisani, P., Ferlay, J. (1999). Estimates of the world-wide incidence of 25 major cancers in 1990. Int. J. Cancer 80, 827-841.

Pasquali, R., Anconetani, B., Chattat, R., Biscotti, M., Spinucci, G., Casimirri, F., Vicennati, V., Carcello, A., Labate, A.M. (1996). Hypothalamic-pituitary-adenal axis activity and its relationship to the autonomic nervous system in women with visceral and subcutaneous obesity: Effects of the corticotropin-releasing factor / anginine-vasopressin test. Metabolism 45, 351-356.

Pasquali, R., Vicennati, V. (2000). Activity of the hypothalamic-pituitary-adrenal axis in different obesity types. Int. J. Obes. Relat. Metab. Disord. 24(Suppl 2), S47-S49.

Payne, S. (1990). Lay representations of breast cancer. Psychology Health 5, 1-11.

Pharoah, P.D.P., Day, N.E., Duffy, S., Easton, D.F., Ponder, B.A.J. (1997). Family history and the risk of breast cancer: A systemic review and meta-analysis. Int. J. Cancer 71, 800-809.

Pike, J.L., Smith, T.L., Hauger, R.L., Nicassio, P.M., Patterson, T.L., McClintick, J., Costlow, C., Irwin, M.R., 1997. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. Psychosom. Med. 59, 447-457.

Powell, L.H., Lovallo, W.R., Matthews, K.A., Meyer, P., Midgley, A.R., Baum, A., Stone, A.A., Underwood, L., McCann, J.J., Janikula Herro, K., Ory, M.G. (2002). Physiologic markers of chronic stress in premenopausal, middle-aged women. Psychosom. Med 64, 502-509.

Prüssner, M., Hellhammer, D.H., Prüssner, J.C., Lupien, S.J. (2003). Self-reported symptoms and stress levels in healthy young men: Associations with the cortisol response to awakening. Psychosom. Med. 65, 92-99.

Rees, G., Fry, A., Cull, A. (2001). A family history of breast cancer: Women's experiences from a theoretical perspective. Soc. Sci. Med. 52, 1433-1440.

Rohleder, N., Schommer, N.C., Hellhammer, D.H., Engel, R., Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. Psychosom. Med. 63, 966-972.

Rohleder, N., Kudielka, B.M., Hellhammer, D.H., Wolf, J.M., Kirschbaum, C. (2002). Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. J. Neuroimmunol. 126, 69-77.

Rohleder, N., Wolf, J.M., Piel, M., Kirschbaum, C. (2003). Impact of oral contraceptive use on glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. Psychoneuroendocrinol. 28(3), 261-273.

Rose, R.M., Jenkins, C.D., Hurst, M., Livingston, L., Hall, R.P. (1982). Endocrine activity in air traffic controllers at work. I. Characterization of cortisol and growth hormone levels during the day. Psychoneuroendocrinol. 7, 101-111.

Rosenstock, I.M. (1966). Why people use health services. Milbank Memorial Fund Quaterly 44, 94-127.

Roy, M.P., Steptoe, A., Kirschbaum, C. (1998). Life events and social support as moderators of individual differences in cardiovascular and cortisol reactivity. J Pers Soc Psychol 75, 1273-1281.

Ruiz, M., Lind, U., Gafvels, M., Eggertsen, G., Carlsted-Duke, J., Nilsson, L., Holtmann, M., Stierna, P., Wikström, A.C., Werner, S. (2001). Characterization of two novel mutations in the glucocorticoid receptor gene in patients with primary cortisol resistance. Clin. Endocrinol. 55, 363-371.

Sandin, B., Chorot, P., Valiente, R.M., Lostao, L., Santed, M.A. (2002). Adverse psychological effects in women attending a second-stage breast cancer screening. J. Psychosom. Res. 52, 303-309.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21(1), 55-89.

Schulz, K.H., Gold, S. (1999). Zur Bedeutung der Psychoneuroimmunologie für die Psycho-Onkologie. Psychomed 11, 198-206.

Selye, S. (1976). Stress in health and disease. Boston: Butterworth.

Sgoutas-Emch, S.A., Cacioppo, J.T., Uchino, B.N., Malarkey, W., Pearl, D., Kiecolt-Glaser, J.C. (1994). The effects of an acute psychological stressor on cardiovascular, endocrine and cellular immune response: A prospective study of individuals high and low in heart rate reactivity. Psychophysiol. 31, 264-271.

Sluiter, J.K., van der Beek, A.J., Frings-Dresen, M.H. (1998). Work stress and recovery measured by urinary catecholamines and cortisol excretion in long distance coach drivers. Occup. Environ. Med. 55, 407-13.

Sluiter, J.K., Frings-Dresen, M.H., van der Beek, A.J. (2000). A forward-facilitating influence of cortisol on catecholamines assessed during the work of garbage collectors. Scand. J. Work Environ. Health 26, 26-31.

Stein-Behrens, B., Mattson, M.P., Chang, I., Yeh, M., Sapolsky, R (1994). Stress exacerbates neuron loss and cytoskeletal pathology in the hippocampus. J. Neurosci. 14, 5373-80.

Sterling, P., Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In: Fisher, S., Reason, J. (Eds.). Handbook of life stress, cognition and health. New York: Wiley, pp. 629-649.

Sternberg, E.M. (2001). The balance within: The science connecting health and emotions. New York: Freeman.

Stout, S.C., Kilts, C.D., Memroff, C.B. (1995). Neuropeptides and stress: Preclinical findings and implications for pathophysiology. In: Friedmann, M.J., Charney, D.S., Deutch, A.Y. Neurobiological and clinical consequences of stress. Philadelphia: Lippincott-Raven.

Symington, T., Currie, A.R., Curran, R.S., Davidson, J.N. (1955). The reaction of the adrenal cortex in conditions of stress. Ciba Foundations Colloquia on Endocrinology, Vol. 8. Boston: Little-Brown.

Theriault, A., Boyd, E., Harrap, S.B., Hollenberg, S.M., Connor, J.M. (1989). Regional chromosomal assignment of the human glucocorticoid receptor gene to 5q31. Hum. Genet. 83, 289-291.

Tversky, A., Kahneman, D. (1974). Judgement under uncertainty: Heuristics and biases. Science 185, 1124-1131.

Ursin, H. (1988). Expectancy and activation: An attempt to sytematize stress theory. In: Hellhammer, D., Florin, I., Weiner, H. (Eds.). Neurobiological approaches to human disease. Toronto: Huber, pp. 313-334.

Valdimarsdottir, H.B., Bovbjerg, D.H., Kash, K.M., Holland, J.C., Osborne, M.P., Miller, D.G. (1995). Psychological distress in women at familial risk of breast cancer. Psycho-Oncology 4, 133-141.

Valdimarsdottir, H.B., Zakowski, S.G., Gerin, W., Mamkos, J., Pickering, T., Bovbjerg, D.H. (2002). Heightened psychobiological reactivity to laboratory stressors in healthy women at familial risk of breast cancer. J Behav Med 25, 51-65.

Van Eck, M.M.M., Nicolson, N.A., Berkhof, H., Sulon, J. (1996). Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. Biol Psychol 43, 69-84.

Vicinnati, V., Pasquali, R. (2000). Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: Evidence for a central and a peripheral alteration. J. Clin. Endocrinol. Metab. 85, 4093-4098.

Ward, M.M., Mefford, I.N., Parker, S.D., Chesney, M.A., Barr Taylor, C., Keegan, D.L., Barchas, J.D. (1983). Epinephrine and norepinephrine responses in continously collected human plasma to a series of stressors. Psychosom. Med. 45, 471-486.

Webster, J.I., Tonelli, L., Sternberg, E.M. (2002). Neuroendocrine regulation of immunity. Ann. Rev. Immunol. 20, 125-163.

Weinstock, M., Poltrev, T., Schorer-Apelbaum, D., Men, D., McCarty, R. (1998). Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. Physiol. Behav. 64(4), 439-444.

Weizman, R., Laor, N., Barber, Y., Selman, A., Schujovizky, A., Wolmer, L., Laron, Z., Gil-Ad, I. (1994). Impact of the Gulf War on the anxiety, cortisol, and growth hormone levels of Israeli civilians. Am. J. Psychiatry 151, 71-75.

Wellisch, D.K., Gritz, E.R., Schain, W., Wang, H.J., Siau, J. (1991). Psychological functioning of daughters of breast cancer patients. Part I. Daughters and comparison subjects. Psychosomatics 32, 324-336.

Wellisch, D.K., Lindberg, N.M. (2001). A psychological profile of depressed and nondepressed women at high risk for breast cancer. Psychosomatics 42, 330-336.

Whiteside, T.L., Herberman, R.B. (1995). The role of natural killer cells in immune surveillance of cancer. Curr. Opin. Immunol. 7, 704-710.

Williams, R.B., Suarez, E.C., Kuhn, C.M., Zimmerman, E.A., Schanberg, S.M. (1991). Biobehavioral basis of coronoary-prone behavior in middle-aged men. I. Evidence for chronic SNS activation in type As. Psychosom. Med. 53, 517-527.

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. Psychopharmacol. (Berlin) 134, 319-329.

Wingo, P.A., Ory, H.W., Layde, P.M., Lee, N.C. (1988). The evaluation of the data collection process for a multicenter, population-based case-control design. Am. J. Epidemiol. 128, 206-217.

WHO (1997). Obesity: Preventing the global epidemic: Report of a WHO consultation of obesity. WHO: Geneva.

Wolf, O.T., Convit, A., McHugh, P.F., Kandil, E., Thorn, E.L., De Santi, S., McEwen, B.S., de Leon, M.J. (2001). Cortisol differentially affects memory in young and elderly men. Behav. Neurosci. 115, 1002-1011.

Wooster, R., Bignelli, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., Collins, N., Gregory, S., Gumbs, C., Mickelm, G. (1995). Identification of the breast cancer susceptibility gene BRCA2. Nature 378, 789-792.

Wüst, S., Federenko, I., Hellhammer, D.H., Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinol. 25, 707-720.

Wüst, S., van Rossum, L., Koper, J.W., Federenko, I., Kumsta, R., Hellhammer, D.H. (2002). Impact of two polymorphisms of the glucocorticoid receptor gene on hypothalamus-pituitary-adrenal axis responses to pharmacological and psychosocial stimulation.[Abstract] Psychosom Med. 64, 129. Presented at the Annual Meeting of the American Psychosomatic Society, Barcelona, Spain, March 2002.

Yamada, K., Duong, D.T., Scott, D.K., Wang, J.C., Granner, D.K. (1999). CCAAT/Enhancer-binding protein is an accessory factor for the glucocorticoid response from the cAMP response element in the rat phosphoenolpyruvate carboxykinase gene promoter. J. Biol. Chem. 274, 5880-5887.

Yang, Y., Koh, D., Ng, V., Lee, F.C., Chan, G., Dong, F., Chia, S.E. (2001). Salivary cortisol levels and work-related stress among emergency department nurses. J. Occup. Environ. Med. 43(12), 1011-1018.

Young, E.A., Akana, S., Dallman, M.F. (1990). Decreased sensitivity to glucocorticoid fast feedback in chronically stressed rats. Neuroendocrinol. 51, 536-42.

Young, A.H., Sahakian, B.J., Robbins, T.W., Cowen, P.J. (1999). The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. Psychopharmacol. 145, 260-266.

Zakowski, S.G., Validmarsdottir, H.B., Bovbjerg, D.H., Borgen, P., Holland, J., Kash, K., Miller, D., Mitnick, J., Osborne, M., Van Zee, K. (1997). Predictors of intrusive thoughts and avoidance in women with family histories of breast cancer. Ann. Behav. Med. 19, 362-369.

Zorilla, E.P., Luborsky, L., McKay, J.P., Rosenthal, R., Houldin, A., Tax, A., McCorkle, R., Seligman, D.A., Schmidt, K. (2001). The relationship of depression and stressors to immunological assays: A meta-analytic review. Brain Behav. Immun. 15, 199-226.

APPENDIX A

Tables A-N

Table A: Distribution of sociodemographic variables of the sample (absolute numbers, total numbers smaller than 83 due to missing data). All group differences are not significant (see text)

Variable		FR- Control	FR- Stress	FR+ Stress
Ethnicity	White (non-Hispanic)	21	23	16
	African American	4	6	1
	Other	3	6	0
Marital status	Never married	17	25	10
	Currently married	6	4	6
	Separated or divorced	5	5	1
Living arrangement	Live alone	9	20	6
	With room-mate	8	2	2
	With spouse or partner	8	9	6
	With parents or other	3	4	3
How long in current living arrangement?	Less than 1 month	2	0	0
	1-6 month	3	6	3
	7 months – 2 years	9	7	5
	2-5 years	8	15	3
	More than 5 years	6	7	6
Education	High School graduate or partial college	1	3	2
	College graduate	17	18	9
	Graduate professional training	10	14	6
Employment	Full time at job	13	18	11
	Part time at job	8	11	3
	Other (3, 4, 5, 6, 9, 11)	7	6	2
Income (US$)	Less than $10,000	2	3	1
	$10,000-$19,999	3	8	2
	$20,000-$39,999	14	12	2
	$40,000-$59,999	3	7	4
	$60,000-$100,000	2	2	3
	Greater than $100,000	4	3	4

Table B: Self-report data on duration of sleep and smoking the days prior to the experiment (visit 2). Sleep data is given in hours per night (mean ± standard deviation), smoking refers to number of cigarettes per day (mean ± standard deviation).

		FR- Control	FR- Stress	FR+ Stress	ANOVA
Sleep last night	Mean + SD	7.11 ± 1.40	6.57 ± 1.39	6.94 + 0.75	F(2; 77)=1.41
	Minimum	2	3	6	p=.25
	Maximum	9	9	8	
Sleep two nights ago	Mean + SD	6.93 ± 1.68	7.32 ± 1.41	7.47 ± 1.23	F(2; 76)=0.87
	Minimum	4	3	6	p=.42
	Maximum	10	10	9	
Sleep three nights ago	Mean + SD	7.36 ± 1.42	7.03 ± 1.29	7.18 ± 1.38	F(2; 76)=0.45
	Minimum	5	4	5	p=.64
	Maximum	10	9	10	
Smoke today	Mean + SD	0.19 ± 0.68	0.17 + 0.51	0.18 + 0.53	F(2; 76)=0.04
	Minimum	0	0	0	p=.99
	Maximum	3	2	2	
Smoke yesterday	Mean + SD	2.07 ± 4.68	1.15 ± 2.69	0.76 ± 2.49	F(2; 75)=0.87
	Minimum	0	0	0	p=.42
	Maximum	20	10	10	
Smoke 2 days ago	Mean + SD	2.52 ± 4.94	2.97 ± 2.43	1.12 ± 2.87	F(2; 75)=1.55
	Minimum	0	0	0	p=.21
	Maximum	20	10	10	
Smoke 3 days ago	Mean + SD	1.41 ± 2.74	1.26 ± 3.08	1.06 ± 2.68	F(2; 75)=0.08
	Minimum	0	0	0	p=.93
	Maximum	10	12	10	

Table C: Self-report data on caffeine and alcohol intake the days prior to the experiment (visit 2). Data is given in cups of coffee per day (mean ± standard deviation) number of alcoholic beverages per day (mean ± standard deviation).

		FR- Control	FR- Stress	FR+ Stress	ANOVA
Caffeine today	Mean + SD	1.61 ± 0.74	1.44 ± 0.56	1.71 ± 0.67	F(2; 76)=1.05
	Minimum	0	0	0	p=.35
	Maximum	2	2	2	
Caffeine yesterday	Mean + SD	1.71 ± 1.43	1.41 ± 1.02	1.59 ± 1.32	F(2; 77)=0.50
	Minimum	0	0	0	p=.61
	Maximum	5	4	5	
Caffeine 2 days ago	Mean + SD	1.39 ± 1.32	1.29 ± 1.13	1.65 ± 1.50	F(2; 77)=0.46
	Minimum	0	0	0	p=.64
	Maximum	4	4	4	
Caffeine 3 days ago	Mean + SD	1.39 ± 1.17	1.32 + 1.16	1.82 + 1.75	F(2; 77)=0.84
	Minimum	0	0	0	p=.44
	Maximum	3	4	6	
Alcohol today	Mean + SD	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N/A
	Minimum	0	0	0	
	Maximum	0	0	0	
Alcohol yesterday	Mean + SD	0.11 ± 0.32	0.43 ± 0.74	0.06 ± 0.26	F(2; 77)=3.98
	Minimum	0	0	0	p=**.02**
	Maximum	1	3	1	
Alcohol 2 days ago	Mean + SD	0.89 ± 1.40	0.34 ± 0.64	0.59 ± 1.18	F(2; 77)=2.04
	Minimum	0	0	0	p=.14
	Maximum	5	2	4	
Alcohol 3 days ago	Mean + SD	0.68 ± 1.12	0.26 ± 0.66	0.56 ± 1.22	F(2; 77)=1.76
	Minimum	0	0	0	p=.18
	Maximum	4	3	4	

Table D: Raw values of epinephrine, norepinephrine, and cortisol at baseline, in response to the stressor (15'), as well as during recovery (30', 45'). Concentrations are given in pg/ml (epinephrine, norepinephrine) and µg/dl (cortisol).

		Baseline	15'	30'	45'
Epinephrine	Stress FR+	66.8 ± 66.8	84,51 ± 84.5	88,12 ± 88.1	75,45 ± 75.5
	Stress FR-	82.7 ± 101.0	94.9 ± 103.1	88.2 ± 99.2	88.9 + 108.1
	Control FR-	114.1 ± 111.4	99,78 ± 94.9	96,99 ± 88.2	106,64 ± 88.9
Norepinephrine	Stress FR+	344.2 ± 136.9	376.3 ± 133.2	346.7 ± 116.2	360.3 ± 134.3
	Stress FR-	356.2 ± 283.3	368.6 ± 262.3	380.4 ± 298.1	368.9 ± 292.1
	Control FR-	330.4 ± 228.6	320.0 ± 212.9	354.1 ± 272.3	354.9 ± 278.3
Cortisol	Stress FR+	11.6 ± 4.9	12.5 ± 5.3	11.6 ± 4.6	10.3 ± 4.5
	Stress FR-	13.1 ± 4.7	12.3 ± 5.0	12.0 ± 4.9	10.8 ± 4.6
	Control FR-	15.6 ± 11.6	13.6 ± 9.9	12.3 ± 8.9	11.1 ± 8.2

Table E: Correlations of self-report measures and acute responses of endocrine measures (delta scores, 15' post baseline – baseline).

		Epinephrine acute response	Norepinephrine acute response	Cortisol acute response
GSI	Pearson Correlation	-.060	-.181	-.089
	Sig. (2-tailed)	.594	.109	.432
PSS	Pearson Correlation	-.052	-.051	-.109
	Sig. (2-tailed)	.648	.655	.336
BDI	Pearson Correlation	-.005	-.163	-.086
	Sig. (2-tailed)	.966	.150	.447
VAS	Pearson Correlation	-.022	-.019	-.056
	Sig. (2-tailed)	.844	.864	.623
GSI [median split]	Pearson Correlation	.302	.017	.176
	Sig. (2-tailed)	.006	.878	.117
PSS [median split]	Pearson Correlation	.293	.054	.156
	Sig. (2-tailed)	.008	.635	.163
BDI [median split]	Pearson Correlation	.289	.070	.143
	Sig. (2-tailed)	.009	.533	.203
VAS [median split]	Pearson Correlation	.284	.110	.205
	Sig. (2-tailed)	.010	.327	.066

Table F: Correlations of self-report measures and recovery of endocrine measures (delta scores, 30’ post baseline – baseline).

		Epinephrine Recovery 30’	Norepinephrine Recovery 30’	Cortisol Recovery 30’
GSI	Pearson Correlation	-.042	-.135	-.116
	Sig. (2-tailed)	.712	.231	.306
PSS	Pearson Correlation	-.006	-.052	-.085
	Sig. (2-tailed)	.956	.649	.452
BDI	Pearson Correlation	.126	-.119	-.056
	Sig. (2-tailed)	.265	.294	.624
VAS	Pearson Correlation	.002	.004	-.081
	Sig. (2-tailed)	.988	.973	.473
GSI [median split]	Pearson Correlation	.331	-.161	.265
	Sig. (2-tailed)	.003	.151	.017
PSS [median split]	Pearson Correlation	.354	-.091	.227
	Sig. (2-tailed)	.001	.419	.042
BDI [median split]	Pearson Correlation	.366	-.062	.274
	Sig. (2-tailed)	.001	.585	.013
VAS [median split]	Pearson Correlation	.311	-.096	.288
	Sig. (2-tailed)	.005	.392	.009

Table G: Correlations of self-report measures and recovery of endocrine measures (delta scores, 45' post baseline – baseline).

		Epinephrine Recovery 45'	Norepinephrine Recovery 45'	Cortisol Recovery 45'
GSI	Pearson Correlation	-.122	-.060	-.100
	Sig. (2-tailed)	.281	.596	.378
PSS	Pearson Correlation	-.105	-.044	-.096
	Sig. (2-tailed)	.355	.701	.396
BDI	Pearson Correlation	-.045	-.129	-.050
	Sig. (2-tailed)	.693	.254	.661
VAS	Pearson Correlation	.030	-.033	-.115
	Sig. (2-tailed)	.795	.769	.310
GSI [median split]	Pearson Correlation	.195	-.162	.197
	Sig. (2-tailed)	.080	.147	.078
PSS [median split]	Pearson Correlation	.203	-.102	.196
	Sig. (2-tailed)	.069	.366	.079
BDI [median split]	Pearson Correlation	.230	-.089	.227
	Sig. (2-tailed)	.039	.431	.041
VAS [median split]	Pearson Correlation	.183	-.129	.210
	Sig. (2-tailed)	.102	.249	.060

Tablle H: VAS-median splits. Raw values of epinephrine, norepinephrine, and cortisol at baseline, in response to the stressor (15'), as well as during recovery (30', 45'). Concentrations are given in pg/ml (epinephrine, norepinephrine) and µg/dl (cortisol).

		Baseline	15'	30'	45'
Epinephrine	Stress VAS-low	66.3 ± 69.6	81.6 ± 78.4	75.8 ± 86.5	69.6 ± 74.3
	Stress VAS-high	91.4 ± 108.2	104.1 ± 107.1	103.4 ± 98.7	102.3 + 110.4
	Control	114.1 ± 111.4	99,78 ± 94.9	96,99 ± 88.2	106,64 ± 88.9
Norepinephrine	Stress VAS-low	329.4 ± 361.0	361 ± 171.7	369.1 ± 190.4	366.3 ± 166.8
	Stress VAS-high	361.0 ± 312.2	373.4 ± 272.9	361.2 ± 308.6	355.7 ± 316.6
	Control	330.4 ± 228.6	320.0 ± 212.9	354.1 ± 272.3	354.9 ± 278.3
Cortisol	Stress VAS-low	12.3 ± 5.6	12.0 ± 5.6	11.2 ± 5.1	10.5 ± 5.2
	Stress VAS-high	13.1 ± 3.9	12.8 ± 4.5	12.6 ± 4.5	11.0 ± 3.9
	Control	15.6 ± 11.6	13.6 ± 9.9	12.3 ± 8.9	11.1 ± 8.2

Tablle I: GSI-median splits. Raw values of epinephrine, norepinephrine, and cortisol at baseline, in response to the stressor (15'), as well as during recovery (30', 45'). Concentrations are given in pg/ml (epinephrine, norepinephrine) and µg/dl (cortisol).

		Baseline	15'	30'	45'
Epinephrine	Stress GSI-low	67.5 ± 82.7	80.9 ± 72.9	74.8 ± 78.7	69.2 ± 62.2
	Stress GSI-high	89.4 ± 98.4	103.8 ± 109.6	103.2 ± 104.0	101.4 + 116.1
	Control	114.1 ± 111.4	99,78 ± 94.9	96,99 ± 88.2	106,64 ± 88.9
Norepinephrine	Stress GSI-low	338.6 ± 139.9	393.3 ± 161.9	396.2 ± 173.2	384.7 ± 147.2
	Stress GSI-high	351.3 ± 309.8	343.2 ± 276.7	336.4 ± 311.3	339.1 ± 319.6
	Control	330.4 ± 228.6	320.0 ± 212.9	354.1 ± 272.3	354.9 ± 278.3
Cortisol	Stress GSI-low	12.4 ± 5.2	12.4 ± 5.1	11.6 ± 4.5	10.8 ± 5.1
	Stress GSI-high	13.0 ± 4.5	12.4 ± 5.1	12.2 ± 5.1	10.7 ± 4.0
	Control	15.6 ± 11.6	13.6 ± 9.9	12.3 ± 8.9	11.1 ± 8.2

Tablle J: PSS-median splits. Raw values of epinephrine, norepinephrine, and cortisol at baseline, in response to the stressor (15'), as well as during recovery (30', 45'). Concentrations are given in pg/ml (epinephrine, norepinephrine) and µg/dl (cortisol).

		Baseline	15'	30'	45'
Epinephrine	Stress PSS-low	80.0 ± 90.5	94.35 ± 93.0	85.3 ± 93.3	81.4 ± 79.6
	Stress PSS-high	77.6 ± 93.2	91.3 ± 96.1	94.1 ± 94.2	90.8 + 110.1
	Control	114.1 ± 111.4	99,78 ± 94.9	96,99 ± 88.2	106,64 ± 88.9
Norepinephrine	Stress PSS-low	300.9 ± 167.3	344.5 ± 190.9	338.4 ± 208.4	330.6 ± 187.5
	Stress PSS-high	393.1 ± 297.9	391.9 ± 264.2	394.1 ± 297.0	393.8 ± 305.3
	Control	330.4 ± 228.6	320.0 ± 212.9	354.1 ± 272.3	354.9 ± 278.3
Cortisol	Stress PSS-low	13.2 ± 5.4	13.4 ± 5.8	12.8 ± 5.6	11.6 ± 5.5
	Stress PSS-high	12.1 ± 4.1	11.4 ± 3.9	10.9 ± 3.5	9.8 ± 3.0
	Control	15.6 ± 11.6	13.6 ± 9.9	12.3 ± 8.9	11.1 ± 8.2

Tablle K: BDI-median splits. Raw values of epinephrine, norepinephrine, and cortisol at baseline, in response to the stressor (15'), as well as during recovery (30', 45'). Concentrations are given in pg/ml (epinephrine, norepinephrine) and µg/dl (cortisol).

		Baseline	15'	30'	45'
Epinephrine	Stress BDI-low	69.0 ± 8.9	84.5 ± 74.9	74.1 ± 75.0	68.6 ± 62.5
	Stress BDI-high	88.5 ± 98.9	101.7 ± 111.2	106.3 ± 108.2	104.7 + 118.7
	Control	114.1 ± 111.4	99,78 ± 94.9	96,99 ± 88.2	106,64 ± 88.9
Norepinephrine	Stress BDI-low	372.3 ± 291.8	412.3 ± 258.7	402.8 ± 300.8	399.1 ± 298.2
	Stress BDI-high	316.0 ± 172.3	318.8 ± 182.5	324.5 ± 188.7	319.9 ± 183.5
	Control	330.4 ± 228.6	320.0 ± 212.9	354.1 ± 272.3	354.9 ± 278.3
Cortisol	Stress BDI-low	14.0 ± 5.6	14.2 ± 5.6	13.1 ± 4.9	12.0 ± 4.9
	Stress BDI-high	11.3 ± 3.4	10.4 ± 3.6	10.6 ± 4.4	9.4 ± 3.6
	Control	15.6 ± 11.6	13.6 ± 9.9	12.3 ± 8.9	11.1 ± 8.2

Table L: Effects of self-report measures on epinephrine resting (control group) levels. Time and Time x Group effects of repeated measures ANOVAs are displayed. Subjects in the control condition were divided based on their score in the respective self-report measures GSI, PSS, BDI, and VAS baseline (median split).

Variable	Effect	F	df	p
GSI [median split]	Time	1.53	3; 24	.23
	Time x Group	0.88	3; 24	.46
PSS [median split]	Time	1.59	3; 24	.22
	Time x Group	0.78	3; 24	.52
BDI [median split]	Time	1.70	3; 24	.19
	Time x Group	1.45	3; 24	.25
VAS [median split]	Time	1.63	3; 24	.21
	Time x Group	0.70	3; 24	.56

Table M: Effects of self-report measures on norepinephrine resting (control group) levels. Time and Time x Group effects of repeated measures ANOVAs are displayed. Subjects in the control condition were divided based on their score in the respective self-report measures GSI, PSS, BDI, and VAS baseline (median split).

Variable	Effect	F	df	p
GSI [median split]	Time	1.03	3; 24	.40
	Time x Group	0.49	3; 24	.69
PSS [median split]	Time	0.98	3; 24	.42
	Time x Group	1.40	3; 24	.27
BDI [median split]	Time	0.89	3; 24	.46
	Time x Group	0.46	3; 24	.71
VAS [median split]	Time	1.07	3; 24	.38
	Time x Group	0.67	3; 24	.60

Table N: Effects of self-report measures on cortisol resting (control group) levels. Time and Time x Group effects of repeated measures ANOVAs are displayed. Subjects in the control condition were divided based on their score in the respective self-report measures GSI, PSS, BDI, and VAS baseline (median split).

Variable	Effect	F	df	p
GSI [median split]	Time	8.77	3; 24	.001
	Time x Group	0.63	3; 24	.60
PSS [median split]	Time	9.35	3; 24	.001
	Time x Group	1.44	3; 24	.26
BDI [median split]	Time	8.21	3; 24	.001
	Time x Group	0.36	3; 24	.78
VAS [median split]	Time	9.58	3; 24	.001
	Time x Group	1.21	3; 24	.33

APPENDIX B

Questionnaires

Visual analog scales

Brief Symptom Inventory

Perceived Stress Scale

Beck Depression Inventory

FOR THE NEXT SERIES OF QUESTIONS,

PLEASE THINK ABOUT HOW YOU ARE FEELING

FOR ANY REASON

RIGHT NOW

How anxious are you feeling RIGHT NOW?

Please put a slash through this line to indicate how anxious you feel.

Not Anxious
At All

As Anxious
As I Could Be

How fatigued are you feeling RIGHT NOW?

Please put a slash through this line to indicate how fatigued you feel.

Not Fatigued At All

As Fatigued As I Could

How confused are you feeling RIGHT NOW?

Please put a slash through this line to indicate how confused you feel.

Not Confused At All

As Confused As I Could

How depressed are you feeling RIGHT NOW?

Please put a slash through this line to indicate how depressed you feel.

Not Depressed At All

As Depressed As I Could

_____

How angry are you feeling RIGHT NOW?

Please put a slash through this line to indicate how angry you feel.

Not Angry
At All

As Angry
As I Could

How tense are you feeling RIGHT NOW?

Please put a slash through this line to indicate how tense you feel.

Not Tense
At All

As Tense
As I Could

How relaxed are you feeling RIGHT NOW?

Please put a slash through this line to indicate how relaxed you feel.

Not Relaxed At All

As Relaxed As I Could

How frustrated are you feeling RIGHT NOW?

Please put a slash through this line to indicate how frustrated you feel.

Not Frustrated At All

As Frustrated As I Could

How nervous are you feeling RIGHT NOW?

Please put a slash through this line to indicate how nervous you feel.

Not Nervous At All

As Nervous As I Could

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
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

	Not at all	A little bit	Moderately	Quite a bit	Extremely
28. Feeling afraid to travel on buses, subways, or trains	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
52. Feelings of guilt	1	2	3	4	5
53. The idea that something is wrong with your mind	1	2	3	4	5

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last week. In each case you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives and circle the corresponding number:

0 = never
1 = almost never
2 = sometimes
3 = fairly often
4 = very often

1. In the last week, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last week, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last week, how often have you felt nervous and stressed?	0	1	2	3	4
4. In the last week, how often have you dealt successfully with irritating life hassles?	0	1	2	3	4
5. In the last week, how often have you felt that that you were effectively coping with important changes that were occurring in your life?	0	1	2	3	4
6. In the last week, how often have felt confident about your ability to handle your personal problems?	0	1	2	3	4
7. In the last week, how often have you felt that things were going your way?	0	1	2	3	4
8. In the last week, how often have you found that you could not cope with all the things you had to do?	0	1	2	3	4
9. In the last week, how often have you been able to control irritations in your life?	0	1	2	3	4
10. In the last week, how often have you felt you were on top of things?	0	1	2	3	4
11. In the last week, how often have you been angered because of things that happened that were outside of your control?	0	1	2	3	4
12. In the last week, how often have you found yourself thinking about things that you have accomplished?	0	1	2	3	4
13. In the last week, how often have you been able to control the way you spend your time?	0	1	2	3	4
14. In the last week, how often have you felt difficulties were piling up so high you could not overcome them?	0	1	2	3	4

BDI

On this questionnaire are groups of statements. Please read each group of statements carefully. Then pick out the one statement in each group which best describes the way you have been feeling for the **PAST WEEK, INCLUDING TODAY**. Circle the number beside the statement you picked. If several statements in the group seem to apply equally well, circle each one. Be sure to read all the statements in each group before making your choice.

A) 0 I do not feel sad.
1 I feel sad.
2 I am sad all the time and I can't snap out of it.
3 I am so sad or unhappy that I can't stand it.

B) 0 I am not particularly discouraged about the future.
1 I feel discouraged about the future.
2 I feel I have nothing to look forward to.
3 I feel that the future is hopeless and things cannot improve.

C) 0 I do not feel like a failure.
1 I feel I have failed more than the average person.
2 As I look back on my life, all I can see is a lot of failures.
3 I feel I am a complete failure as a person.

D) 0 I get as much satisfaction out of things as I used to.
1 I don't enjoy things the way I used to.
2 I don't get real satisfaction out of anything any more.
3 I am dissatisfied or bored with everything.

E) 0 I don't feel particularly guilty.
1 I feel guilty a good part of the time.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

F) 0 I don't feel disappointed in myself.
1 I am disappointed in myself.
2 I am disgusted with myself.
3 I hate myself.

G) 0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

H) 0 I don't feel I am any worse than anybody else.
1 I am critical of myself for my weaknesses or mistakes.
2 I blame myself all the time for my faults
3 I blame myself for everything bad that happens.

I) 0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

J) 0 I don't cry any more than I used to.
1 I cry more now than I used to.
2 I cry all the time now.
3 I used to be able to cry but now I can't cry even though I want to.

K) 0 I am no more irritated now than I ever was.
1 I get annoyed or irritated more easily than I used to.
2 I feel irritated all the time now.
3 I don't get irritated at all by the things that used to irritate me.

L) 0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all my interest in other people.

M) 0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty making decisions than I used to.
3 I can't make decisions at all any more.

N) 0 I can work about as well as before.
1 It takes extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

O) 0 I don't feel I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive.
3 I believe I look ugly.

P) 0 I can sleep as well as I used to.
1 I don't sleep as well as I used to.
2 I wake up earlier than I used to and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

Q) 0 I don't get any more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

R) 0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all any more.

S) 0 I haven't lost much weight, if any, lately.
1 I have lost more than 5 pounds.
2 I have lost more than 10 pounds.
3 I have lost more than 15 pounds.
(On a diet: NO___; YES___)

T) 0 I am no more worried about my health than usual.
1 I am worried about physical problems such as aches and pains, upset stomach, and constipation.
2 I am very worried about physical problems and it is hard to think about much else.
3 I am so worried about my physical problems, I cannot think about anything else.

U) 0 I have not noticed any recent changes in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex than I used to be.
3 I have lost interest in sex completely.