ANXIETY AND AUTO ANTIBODY PRODUCTION TO HEAT SHOCK PROTEIN 70 IN PATIENTS UNDERGOING SURGERY FOR SUSPECTED ENDOMETRIAL CANCER

By

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To my father
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Heat shock proteins (HSPs) are a class of chaperone proteins that protect cells from various forms of stress. Prior work has linked increased psychological stress to increased expression of HSPs in animal models and, more recently, in humans. This study investigated whether greater stress, depression, and anxiety were associated with greater HSP70 among women undergoing surgery for endometrial cancer. In a secondary aim, hypothalamic-pituitary-adrenal (HPA) functioning (i.e., salivary cortisol, area under the curve [AUCg]) was explored as a mediator of the relationship between psychological variables and HSP70. Participants were 38 women attending a Gynecologic Oncology clinic for evaluation of endometrial cancer. Participants underwent pre-surgical psychological assessment with the Life Experiences Survey (LES) and the Structured Interview Guide for the Hamilton Anxiety and Depression Scales (SIGH-AD) and a peripheral venous blood draw. HSP70 antibody concentrations were assessed using enzyme linked immunosorbent assay (ELISA). Controlling for biobehavioral confounds (ie. age, body mass index), neither greater impact of negative life events nor depression was associated with HSP70 ($\beta=.28, p=.099; \beta = .23, p=.16$; respectively). However, a marginally significant association emerged between greater anxiety and greater HSP70 ($\beta=.32, p=.055$), which accounted for 9.1% of the variance in HSP70 above and beyond relevant biobehavioral control.
variables. Cortisol (AUCg) was then examined as a mediator of this relationship. Cortisol was related to pre-operative anxiety ($\beta=.31, p=.035$). However, cortisol was not significantly related to HSP70 when controlling for pre-operative anxiety; thus, cortisol failed to emerge as a mediator. Although based on a small sample, these findings suggest a moderate to large effect size between anxiety and serum HSP70 antibody concentration and anxiety and pre-operative cortisol in women undergoing surgery for endometrial cancer. Future research should examine other measures of HPA activation act as mediators of this relationship in a larger sample, as well as examining additional mood states and HSP protein expression in women with gynecologic cancers.
CHAPTER 1
INTRODUCTION

Heat Shock Proteins

Heat shock proteins (HSPs) are characterized as a class of ubiquitously expressed chaperone proteins, which assist in specific intracellular and extracellular processes such as protein-protein interactions. These proteins receive their name from their ability to protect the cell from extracellular stressors such as inflammation, infection or toxic exposures (Mosser, Caron, Bourget, Denis-Larose, & Massie, 1997) and therefore have come to be referred to as “stress proteins” (Marber et al., 1995; Mestril, Chi, Sayen, O’Reilly, & Dillmann, 1994). HSPs, labeled according to their molecular weight in kilodaltons such as HSP70 or HSP90, protect cells by protect cells by assisting misfolded proteins to fold correctly or by targeting them for removal. In this way, they play critical roles in the intracellular trafficking of protein expression (Asea, 2003). While the exact physiologic role of these highly conserved proteins may still not be fully appreciated, it is thought that they aid in maintaining cellular homeostasis. They have recently been shown to have immunomodulatory properties, acting as both cytokine and chaperone molecules (Asea, 2003; Asea et al., 2000).

HSPs and Cancer

Given the critical role of HSPs in cell protection and apoptosis, many studies have focused on the role of HSPs in carcinogenesis. HSP overexpression has been observed within a number of different cancer cell lines, and extracellular levels of HSP may also influence cancer progression (for review see (Ciocca & Calderwood, 2005)). HSPs role in adaptive immunity has been exploited for novel cancer immunotherapy treatments (Tamura, Peng, Liu, Daou, & Srivastava, 1997; X. Y. Wang, Kaneko, Repasky, & Subjeck, 2000). In these investigations, HSPs were engineered from patients’ cancer cells and injected back into the patient, taking
advantage of HSPs unique roll in the body’s adaptive immune system by allowing the patient’s T cells to recognize the tumor cells within the body.

One possible linkage between extracellular HSPs and carcinogenesis is through HSPs involvement in the inflammatory process, which is known to influence cancer development and metastasis (Coussens & Werb, 2002; Pikarsky et al., 2004). Recent experimental evidence demonstrates that HSP bind to critical immune cells (monocytes), which in turn release potent inflammatory signaling molecules, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 (Asea et al., 2000). These molecules are associated with chronic inflammation, which is linked with a host of disease states such as diabetes, hypertension, and carcinogenesis (Aggarwal, Shishodia, Sandur, Pandey, & Sethi, 2006).

**HSP70 and Endometrial Cancer**

In endometrial cancer, HSP70 has received increasing attention in the evaluation of pathological characteristics of tumors as well as the clinical presentation of patients diagnosed with the disease. In Ciocca and Calderwood’s (2005) review of HSP and cancer, the authors highlight three promising areas for the role of HSPs in cancer progression, all of which are specifically implicated in the progression of endometrial cancer: 1) diagnostic implications (endometrial cancer patients with HSP70-positive tumors have been shown to have poorer survival than those patients with HSP-negative tumors (Nanbu et al., 1998)), 2) carcinogenesis (in endometrial cancer overexpression of HSP70 has been associated with p53 protein expression (Nanbu et al., 1996)) – a known oncogene product) and 3) tumor cell differentiation (overexpression of HSP70 has been associated with a poorly differentiated state in endometrial cancer (Piura, Rabinovich, Yavelsky, & Wolfson, 2002)). Collectively, these data suggest HSPs, particularly HSP70, play a role in the progression of endometrial cancer.
Antibodies to HSP

Similar to HSP expression in extracellular tissue fluids, antibodies to HSPs also fluctuate when the body is exposed to noxious environmental stimuli. Thus high levels of HSP antibodies may represent the aftermath of high levels of circulating HSPs. Elevated antibodies to HSPs (compared to controls) are observed in the peripheral blood of those undergoing noise induced hearing loss (Yang et al., 2004), children with idiopathic thrombocytopenic purpura (Yang et al., 2004), patients undergoing heatstroke (Z. Z. Wang et al., 2001), patients with angina chest pain (Herz, Rosso, Roth, Keren, & George, 2006) and mothers giving birth to babies with birth defects (Child et al., 2006). In particular, antibodies to HSP70 have been observed in the serum of normal individuals (Pockley, Shepherd, & Corton, 1998) as well as in response to disease states and environmental stimuli (Wu & Tanguay, 2006).

Antibodies to HSPs have been observed in cancer populations, and may offer some prognostic value. For example, HSP90 antibodies have been found to be significantly higher in late stage ovarian cancer (Luo, Herrera, Soosaipillai, & Diamandis, 2002). HSP70 antibodies were significantly higher in non-small cell lung cancer patients compared to controls, while HSP90 antibody levels were not (Zhong et al., 2003). Interestingly, relevant antibody levels were not related to tumor stage or histological characteristics in this investigation. Contrary to the supposition that HSP antibodies are physiological harmful, HSP90 antibodies significantly predicted responses to neoadjuvant chemotherapy, whereas their absence was associated with greater lung metastases (although limited by a small sample size, N=3; Trieb et al., 2000). These studies suggest that, while the exact physiologic role of these antibodies is unclear, they are involved in the carcinogenic process.
HSPs and Psychological Stress

HSPs are involved in protecting cells from a number of physiologic processes that carry the potential to damage cellular makeup, one of which is psychological stress. For example, restraint stress, which is known to activate the hypothalamic pituitary adrenal (HPA) axis, induces the expression of HSP70 mRNA in rats (Blake, Udelsman, Feulner, Norton, & Holbrook, 1991). HSP expression is enhanced by HPA-induced glucocorticoid production and by sympathetic nervous system (SNS) induced production of catecholamines (Blake, Klevay, Halas, & Bode, 1995). Extracellular stress, with the addition of proinflammatory cytokines, is also capable of inducing the release of HSP70 from tumor cells (Barreto, Gonzalez, Kabingu, Asea, & Fiorentino, 2003). These studies suggest a relationship between the stress response system, or HPA axis, and HSP production in humans. However, relatively few studies have examined the relationship between psychosocial factors that are known to impact HPA axis functioning and HSP expression and impact in humans.

HPA Functioning and Cortisol Measurement

Given the association between HPA functioning and HSP expression, it may be useful and instructive to examine established measures of HPA functioning when investigating psychological variables and HSPs. One common measure of HPA functioning is quantifying the glucocorticoid cortisol (Kirschbaum & Hellhammer, 1994). Cortisol is produced by the adrenal glands with excitation of the HPA axis, and its release can be measured in the peripheral circulation as well as in salivary excretions. In fact, salivary cortisol is considered an accurate reflection of free (i.e., unbound) cortisol levels in the blood (Kirschbaum & Hellhammer, 1994). Prior research has demonstrated consistent relationships between cortisol and a number of psychological states, including depression (Burke, Davis, Otte, & Mohr, 2005), post traumatic stress disorder (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007), panic disorder (Abelson,
Khan, Liberzon, & Young, 2007) and anxiety (Graeff, 2007). To our knowledge, no research has examined relationships between cortisol and HSP expression in humans, despite the preponderance of evidence suggesting cortisol as a reliable biological marker of HPA functioning and the reported relationships between HPA axis activation and HSP expression (Blake, Buckley, & Buckley, 1993; Blake et al., 1991).

One recent investigation has correlated HSP60 expression with psychological variables in a civil servant population (Lewthwaite, Owen, Coates, Henderson, & Steptoe, 2002). In this study blood samples were obtained from 229 British civil servants who were asked about their socioeconomic status and social isolation. Psychological distress was measured using the 30-item short version of the General Health Questionnaire. In this sample, socioeconomic status was inversely related to plasma HSP60 and high social isolation corresponded to the group with the highest levels of HSP60. Notably, women with the highest levels of stress also exhibited the highest levels of plasma HSP60. Taken together, this data suggest a link between psychological factors and HSP concentrations in peripheral blood in a human population.

**Current Study**

The current study aims to draw a link between previously established relationships between psychological variables, HPA axis dysregulation, and HSP expression in a group of women undergoing treatment for endometrial cancer during the perioperative period. Psychological stress is predicted to be particularly elevated for this group of participants leading up to their surgery, and the biological correlates of this stress (HPA dysregulation, HSP70 expression) are likely to have particular relevance given the link between HPA functioning and cancer (Sephton, Sapolsky, Kraemer, & Spiegel, 2000), as well as HSPs implication in endometrial cancer (Nanbu et al., 1996; Nanbu et al., 1998). In the current study it is hypothesized that high levels of psychological distress around the pre-operative period (high life
stress, depression and anxiety) will be associated with increased levels of circulating antibodies to HSP70, and that this relationship will be mediated by HPA dysregulation (cortisol).
CHAPTER 2
METHODS

Design

The design of the current study was cross sectional and nonexperimental. Participants were women undergoing surgery for suspected endometrial cancer. Participants underwent psychosocial interview and peripheral venous blood draw at their pre-operative clinic visit. The relationships between stressful life events, symptoms of depression, symptoms of anxiety, and auto-antibody levels to HSP70 were then analyzed. Cortisol was examined as a mediator of any significant psychosocial-HSP70 relationships. All analyses controlled for biobehavioral variables which were found to be related to levels of HSP. This study was conducted in accordance with the rules and regulations of the Institutional Review Board at the University of Florida and is IRB approved (project number 69-2004).

Participants

Participants in this study were recruited as part of an ongoing study funded by the American Cancer Society (ACS) and the National Cancer Institute (NCI; R03CA117480-01A1) investigating psychoneuroimmunologic relationships in women undergoing surgery for suspected endometrial cancer. Inclusion criteria included suspected endometrial adenocarcinoma, scheduled to undergo total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO) with or without pelvic lymph node dissection, and fluency in spoken English. Exclusion criteria included recurrent or stage IIIb, IIIc, or IV endometrial carcinoma; pre-surgical chemotherapy or radiation therapy, metastasis to the uterine corpus from another site, current psychotic disorder, or current suicidal intent/plan.
Procedures

Women were recruited prior to undergoing surgery for suspected endometrial cancer from the University of Florida Shands Hospital Gynecology Oncology clinic. Eligibility was determined by brief chart review and physician consultation. Once the patient was deemed eligible following initial screening and agreed to have the researcher speak with her, the patient was presented with relevant study details. If the patient was interested in participating, informed consent was then obtained and a brief additional screening assessment was performed. If the results of this screening were unremarkable, a brief psychosocial interview was scheduled for the participant’s pre-operative appointment at the Gynecology Oncology clinic. Participants were reimbursed $20 at the conclusion of the interview for their time and participation.

Psychosocial Assessment

Pre-surgical Life Stress

Pre-surgical life stress was assessed using the modified and abbreviated Life Experiences Survey (LES; Sarason, Johnson, & Siegel, 1978) in order to assess the number of events deemed stressful over the last six months. The LES is a 60-item measure that assesses the frequency of specific life events over the past 6 months and past 12 months, as well as the perceived impact, chronicity, and controllability of any events that were experienced. The abbreviated version of the LES included in the present study was developed by Leserman and colleagues at the University of North Carolina for use with chronically ill populations. This version was then modified to anchor health related events to the experience of being evaluated for or having cancer. Participants were asked to indicate which events occurred over the prior six months and to rate the degree to which these events were experienced as stressful on a 5 point scale (from “not stressful” to “extremely stressful”). These impact scores were then summed to provide a life stress score, the possible range of which was 0 to 170. Abbreviated versions of LES have
been used in a number of recent studies examining the association between impact of life events, immunity, and disease outcomes among women with chronic and life-limiting medical illnesses (e.g., Pereira et al., 2003). The full LES has shown good internal consistency (Cronbach’s alpha=0.84-0.91), and sufficient test-retest reliability (r=0.80) in cancer patient populations (Thewes, Meiser, & Hickie, 2001).

**Depressive and Anxious Symptomatology**

Depressive and anxious symptomatology were assessed using an abbreviated version of the Structured Interview Guide for the Hamilton Anxiety and Depression scales (SIGH-AD; (Williams, 1988). The SIGH-AD assesses anxious and depressive symptomatology over the past week through a series of structured questions. It has been widely used in patient populations and has adequate reliability and validity in medical settings (Cruess et al., 2000). An abbreviated version of the SIGH-AD was used in order to reduce patient burden and to remove items that would be confounded with endometrial cancer symptomatology (ie. genitourinary symptoms, weight loss). A total of 15 depression items and 9 anxiety items were retained in the present study. Possible depression subscale scores ranged from 0 to 44, while possible anxiety subscale scores ranged from 0 to 29. Higher scores indicated greater symptomatology. Because the version in use in this study was an abbreviated form, depression and anxiety scores from the present study could not be compared to those in other published studies.

**Physiologic Assessment**

**HSP70 Antibody Measurement**

Antibodies to HSP70 were measured by an Enzyme Linked Immunosorbent Assay (ELISA) kit (StressGen, Assay Designs, Ann Arbor, MI). This kit utilized recombinant human HSP70 pre-coated to the wells of the recombinant HSP70 immunoassay plate to capture anti-human HSP70 antibodies present in human serum. The captured anti-human HSP70 antibodies was then
detected with a hydrogen peroxidase conjugated goat polyclonal antibody specific for IgG/A/M antibody molecules. After the substrate produced a readable change in optical density, the color’s intensity of the color is measured in a microplate reader at 450nm. This HSP70 antibody ELISA kit generates a standard curve using a calibrated standard of anti-human HSP70 (IgG/A/M) antibodies isolated from pooled human sera. The resulting predicted values of HSP70 are reported in ng/mL (Figure 1).

Figure 2-1. ELISA curve-fit estimation for HSP70 antibodies

**Salivary Cortisol**

Participants’ collected saliva collection at 8:00, 12:00, 17:00 and 21:00 hours during each of the three days leading up to their pre-operative study visit. Participants were asked to collect saliva
samples using a Salivette (Starstedt, Inc., Newton, N.C.), which is a commercially available conical tube containing a cotton swab. Participants were asked to place the Salivette in their mouth until it was saturated (usually for one or two minutes). Participants were asked to record the time of each saliva collection, to ensure the accuracy of the time points in which the samples were collected. In addition, participants were asked to refrigerate the saliva samples until they were able to bring them to their pre-operative study visit, and they were provided with a small insulated cooler to transport the samples back to the research team. Once received by study personnel, saliva samples were frozen at -70 degrees Celsius by study personnel until they were shipped to Salimetrics Inc. (State College, PA) for assaying.

Cortisol levels were assessed using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (Salimetrics, Inc., State College, PA). This commonly used laboratory technique utilizes a purified version of a substance of interest which is mixed with a specific enzyme in addition to the test sample (containing an unknown amount of the substance of interest). The solution then changes color based on the amount of the substance of interest present. The standard (or known amount of cortisol) and unknown cortisol compete for binding sites on the assay plate. The unbound structures are removed after an incubation period and the bound cortisol produces a reaction with the substrate that can be read by a standard plate reader. Thus, this assay technique assumes that the amount of cortisol is proportional to the intensity of color change read by the plate reader.

Salivary Cortisol Area under the Curve Analyses
Research continues to grow examining relationships between psychosocial factors and cortisol. There are a number up different approaches to measuring cortisol, including early morning cortisol peak, diurnal slope, area under the curve with respect to increase (AUCi), and area under the curve with respect to ground (AUCg; Vedhara, Tuinstra, Miles, Sanderman, & Ranchor,
Based upon the Salivary Cortisol Measurement guidelines outlined by the John D. and Catherine T. MacArthur Research Network on Socioeconomic Status and Health (Stewart & Seeman, 2000), cortisol was operationalized using AUCg in the present study. As outlined in this report, AUCg appears to provide the most accurate and least controversial assessment of basal HPA-axis activity and is thought to correlate the most strongly with psychological functioning. Cortisol AUCg was calculated using a published trapezoidal formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

**Statistical Procedures**

All variables were examined for normal distributions to confirm that parametric statistics were appropriate and if they were found non-normal, the appropriate transformation was applied. Initially, the predicted values of HSP70 were calculated as described above. Next, four potential biobehavioral control variables were investigated for their relationship with HSP70 antibody levels that have been associated with HSP70 antibody levels in prior research (Lewthwaite et al., 2002; Nanbu et al., 1996; Rea, McNerlan, & Pockley, 2001). Biobehavioral variables that were significantly associated with HSP70 at \( p \leq .10 \) were retained as control variables in subsequent regression analyses. Statistics presented here utilized the general linear model (a) to predict circulating HSP70-antibody levels from psychological variables (negative life stress and depressive/anxious symptoms), and (b) to examine cortisol (AUCg) as a mediator of any significant relationships between these psychological variables and HSP70-antibody levels using the methods of Baron and Kenny (1986; Figure 2-2).
Figure 2-2. Cortisol as a mediator between psychological factors and HSP70-antibodies
CHAPTER 3
RESULTS

Demographics

115 women met eligibility criteria and were enrolled in this study. From this group, full psychosocial and HSP-antibody data was collected on 38 participants. T-test and chi square analyses revealed that there were no statistically significant differences (p>0.05) between those who provided full psychosocial and physiologic data and those who did not across age, ethnicity, race or yearly income (Tables 3-1 and 3-2). However, years of education was significantly lower ($t [36]= -2.53; p < .05; Table 3-1$) and total annual income was marginally lower ($t [36]= -1.83; p = .08; Table 3-1$) in the subsample, suggesting that the findings of the present study may not be generalizable to higher socioeconomic status women.

The 38 participants who provided full data ranged in age from 35-84 ($M=61.6, SD=10.9$). The majority of women primarily identified themselves as Caucasian (92.1%) and non-Hispanic (97.4%).

Health Status

The body mass index (BMI) of this select group of women ranged from 20.6 to 55.1 ($M=36.1, SD=10.7$). Five women were overweight (13.2%), while the majority of participants were obese ($n = 23; 67.6$%), defined by a BMI as $\geq 30$ (World Health Organization, 2006, Table 1).

Most participants had Stage I endometrial carcinoma (68.4%), although 13.2% had Stage II disease, and 10.5% had Stage III disease. Two women (5.3%) were found to have benign disease after surgery, and surgical stage was unreported on two others (5.3%).

Tumor grade classifies the severity of the tumor and includes the categories of benign, well-differentiated, moderately differentiated and poorly differentiated. In this sample, roughly
half were classified as well-differentiated (47.4%), with most of the remainder classified as moderately differentiated (36.8%). A small group of patients were classified as benign and poorly differentiated (n=2; 5.3% in each group). Tumor grade was unreported on two participants (5.3%)

**Antibodies to HSP70**

After conducting the curve fit analysis (described above), the resulting predicted values of HSP-antibodies (\(M=241.2\mu g/mL, SD = 202.9\mu g/mL\)) were non-normally distributed, (Skewness = 1.105, SE = .388; Kurtosis = 1.199, SE = .759). Thus, a square root transformation was applied to appropriately reduce skewness and kurtosis (Skewness = .146, SE = .383; Kurtosis = -.574, SE = .750). The range of the square root transformation of antibodies to HSP70 in this sample was from 1.0 to 29.4 (\(M=14.0, SD=6.75\)).

**Investigation of Biobehavioral Control Variables**

Next, the relationship between the four proposed biobehavioral control variables (age, BMI, cancer stage and tumor grade) and HSP-antibodies was examined to select relevant sample-specific control variables. Pearson’s correlations were used to examine the relationships between the continuous predictors (age and BMI) and HSP70. One-way ANOVA’s were conducted to examine relationships between tumor grade, cancer stage and HSP70 (Table 3-3). Age was significantly negatively correlated with HSP70 (\(r =-.32; p = .05\)). BMI was marginally associated with HSP70 (\(r = -.31; p = .073\)). Neither cancer stage (\(F(3,33)=2.92; p=.26\)) nor tumor grade (\(F(3,32)=1.88; p=.15\)) was significantly related with HSP70-antibody levels. Using Pedhazur’s (Pedhazur, 1997) liberal guidelines for at least 15 subjects per predictor in regression analysis, age and BMI were combined into a single predictor in order not to exceed the recommended number of predictors (i.e., 2) for a sample size of 38. As such, an interaction term
was created between age and BMI. This interaction term was highly correlated with HSP70-antibodies ($r = -.48; p = .005$).

**Life Stress**

Life stress scores ranged from 1.0 to 29.0 ($M=8.0, SD=6.0$). The most common life events endorsed in the 6 months prior to surgery were: a major worsening of a financial status (15.8%), having a major illness not related to cancer (15.8%), death of a relative (10.5%), death of a very close friend (10.5%), and working long hours (10.5%).

**Depressive and Anxious Symptoms**

The mean depression subscale score was 5.6 ($SD=4.6$). The mean anxiety subscale score was 4.7 ($SD=5.6$).

**Relationships between Psychological Variables and HSP70-antibodies**

Three hierarchical regression analyses were run to investigate relationships between psychological variables and HSP70-antibodies, all of which included the age/BMI interaction term in the first block as a control variable. In the second block, the psychological variable of interest was entered (i.e., negative life stress, depressive symptomatology, anxious symptomatology).

In the first analysis, negative life stress was entered into the second block. Negative life stress was not a significant predictor of HSP70-antibodies, $\beta = .28, t(27) = 1.71, p = .099$. Furthermore, it explained a nonsignificant (7.9%) amount of variance in HSP70 above and beyond age/BMI (Table 3-4),

In the second analysis, depressive symptomatology was entered into the second block. However, depressive symptoms over the past week also failed to predict HSP70-antibodies $\beta = .23, t(30) = 1.45, p = .16$. Depressive symptoms explained a nonsignificant (5.1%) amount the variance in HSP70 above and beyond age/BMI (Table 3-5).
In the third analysis, anxious symptomatology was entered into the second block. In this instance, greater anxious symptomatology was marginally associated with greater antibodies to HSP70 after controlling for age and BMI, $\beta = .32, t(30) = 2.00, p = .055$ (Table 3-6). Furthermore, pre-operative anxiety explained an additional 9% of the variance above and beyond age/BMI (Total $R^2=.32, F(2,30) = 6.94, p = .003$).

**Cortisol as a Mediator of Pre-operative Anxiety and HSP70**

Given that anxious symptoms were marginally significantly associated with HSP70-antibodies (Table 3-6; Figure 3-1, Path c), exploratory analyses were conducted to examine whether cortisol AUCg mediated the relationship between anxious symptoms and HSP70-antibodies. Raw cortisol AUCg values ranged from 0.46 to 4.96 nmol/l ($M=1.91$ nmol/l, $SD=1.09$ nmol/l). For illustrative purposes, cortisol AUCg for a randomly selected sample of 3 participants is presented in Figure 3-2.

Next, the average AUCg cortisol data were log-transformed to reduce skewness and kurtosis (pre-transformation: Skewness = 0.964, SE = 0.434; Kurtosis = 0.247, SE = 0.845; post-transformation: Skewness = -0.401, SE = 0.434; Kurtosis = 0.123, SE = .845). The age/BMI interaction term was retained as a biobehavioral control variable in all equations.

In order to test Path a, cortisol was regressed on age/BMI (block 1) and anxious symptomatology (block 2). Greater anxious symptomatology was associated with greater cortisol, $\beta = .31, t(44) = 2.18, p = .035$ (Table 3-7; Figure 3-1, Path a) and explained 11% of the variance in HSP70 above and beyond age/BMI.

To test Path b, HSP-70 antibodies were regressed on age/BMI, anxious symptomatology, and cortisol AUCg. However, cortisol AUCg was not a significant predictor of HSP70-antibodies when controlling for age/BMI and pre-operative anxiety, $\beta = .27, t(20) = 1.55, p = .14$ (Table 3-8; Figure 3-1, path b). Thus, path c’ could not be tested.
Figure 3-1. Cortisol as a mediator between psychological factors and HSP70-antibodies
Figure 3-2. Area under the curve with respect to ground (AUCg) analyses for cortisol for three select participants. Area is calculated using the trapezoidal formula for each day and averaged across the three days of collection.
### Table 3-1. Comparisons of selected group for HSP70-antibody assay and the rest of sample

<table>
<thead>
<tr>
<th></th>
<th>Hsp70 Data</th>
<th>No Hsp70 Data</th>
<th>df</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>38</td>
<td>77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>61.58</td>
<td>60.47</td>
<td>37</td>
<td>0.63</td>
<td>.53</td>
</tr>
<tr>
<td>(M (SD))</td>
<td>(10.89)</td>
<td>(8.44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yearly Income ($)</td>
<td>~$20,500</td>
<td>~$26,000</td>
<td>36</td>
<td>-1.83</td>
<td>.08</td>
</tr>
<tr>
<td>(M (SD))</td>
<td>($13,000)</td>
<td>($19,000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education (yrs)</td>
<td>12.75</td>
<td>13.54</td>
<td>36</td>
<td>-2.53</td>
<td>.02</td>
</tr>
<tr>
<td>(M (SD))</td>
<td>(2.61)</td>
<td>(2.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3-2. Comparisons of race for HSP70-antibody assay and the rest of sample

<table>
<thead>
<tr>
<th>Race</th>
<th>Hsp70 Data</th>
<th>No Hsp70 Data</th>
<th>(X^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>White (n)</td>
<td>35</td>
<td>65</td>
<td>.020</td>
<td>.89</td>
</tr>
<tr>
<td>Non-White (African-American) (n)</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3-3. Relationships between HSP70-antibodies and Potential Biobehavioral Control Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with HSP70-antibodies</th>
<th>One way ANOVA F-test (df’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-.32**</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-.31*</td>
<td></td>
</tr>
<tr>
<td>Cancer Stage</td>
<td></td>
<td>1.41 (3, 33)</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td></td>
<td>1.88 (3, 32)</td>
</tr>
<tr>
<td>Age/BMI Interaction Term</td>
<td>-.48***</td>
<td></td>
</tr>
</tbody>
</table>

* \(p \leq .10\)

** \(p \leq .05\)

*** \(p \leq .001\)

### Table 3-4. Predicting HSP70-antibodies from pre-operative life stress

<table>
<thead>
<tr>
<th>Step Number</th>
<th>Predictor Variable</th>
<th>(R^2)</th>
<th>(\beta)</th>
<th>(\Delta R^2)</th>
<th>(F) of (\Delta R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age/BMI</td>
<td>.20</td>
<td>-.44**</td>
<td>.20</td>
<td>6.77**</td>
</tr>
<tr>
<td>2</td>
<td>LES Sum Score</td>
<td>.28</td>
<td>.28*</td>
<td>.08</td>
<td>2.92*</td>
</tr>
</tbody>
</table>

\(n=30\). Significance of Model, \(F(2, 27)=5.08 p=.013\)

* \(p \leq .10\); ** \(p \leq .05\)
Table 3-5. Predicting HSP70-antibodies from symptoms of depression

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor Variable</th>
<th>R²</th>
<th>β</th>
<th>ΔR²</th>
<th>F of ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age/BMI</td>
<td>.23</td>
<td>-.48**</td>
<td>.23</td>
<td>9.03**</td>
</tr>
<tr>
<td>2</td>
<td>SIGH-AD</td>
<td>.28</td>
<td>.23</td>
<td>.05</td>
<td>2.11</td>
</tr>
</tbody>
</table>

n=33. Significance of Model, F(2,30)=5.73, p=.008
* p < .10; **p< .05

Table 3-6. Predicting HSP70-antibodies from symptoms of anxiety

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor Variable</th>
<th>R²</th>
<th>β</th>
<th>ΔR²</th>
<th>F of ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age/BMI</td>
<td>.23</td>
<td>-.48**</td>
<td>.23</td>
<td>9.03**</td>
</tr>
<tr>
<td>2</td>
<td>SIGH-AD</td>
<td>.32</td>
<td>.32^</td>
<td>.09</td>
<td>3.99^</td>
</tr>
</tbody>
</table>

n=33. Significance of Model, F(2,30)=6.94, p=.003
^p=.055, **p< .05

Table 3-7. Path a of mediation analyses: Predicting Cortisol AUCg from symptoms of anxiety

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor Variable</th>
<th>R²</th>
<th>β</th>
<th>ΔR²</th>
<th>F of ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age/BMI</td>
<td>.01</td>
<td>.09</td>
<td>.01</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>SIGH-AD</td>
<td>.11</td>
<td>.31**</td>
<td>.10</td>
<td>4.75**</td>
</tr>
</tbody>
</table>

n=47, Significance of Model, F(2,43)=2.57, p = .09
* p < .10; **p< .05

Table 3-8. Path b of mediation analyses: Predicting HSP70 from symptoms of anxiety and Cortisol AUCg

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor Variable</th>
<th>R²</th>
<th>β</th>
<th>ΔR²</th>
<th>F of ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age/BMI</td>
<td>.31</td>
<td>-.55**</td>
<td>.31</td>
<td>9.65**</td>
</tr>
<tr>
<td>2</td>
<td>Cortisol AUCg</td>
<td>.49</td>
<td>.26</td>
<td>.18</td>
<td>3.57**</td>
</tr>
</tbody>
</table>

n=25, Significance of Model, F(2,23)=6.35, p=.003
* p ≤ .10; **p≤ .05
CHAPTER 4
DISCUSSION

Previous investigators have examined HSP expression and induced psychological stress in laboratory animal models (Blake et al., 1995; Blake et al., 1991), and more recently have extended this research to relationships between HSPs and psychosocial stress in humans (Lewthwaite et al., 2002). No research to our knowledge has examined relationships between psychological variables and antibody production to HSPs in humans. Furthermore, investigating HSP production in a cancer population may have particular significance given the implication of HSP antibody and HSP expression in carcinogenesis (Ciocca & Calderwood, 2005). While the exact clinical relevance of HSP70 in endometrial cancer has yet to be determined, reducing pre-operative anxiety in women undergoing surgery for endometrial cancer may have beneficial effects on HPA functioning (cortisol) as well as reducing circulating antibodies to HSP.

The present study is the first to establish specific relationships between HSPs and anxiety, and the first to examine whether HPA functioning, as measured by cortisol, may potentially mediate this relationship. Endometrial cancer is the most common cancer of the female reproductive system and it is estimated that in 2008, 40,100 women will have been diagnosed and 7,470 will have died from the disease (National Cancer Institute, 2009; Ries et al., 2003). Fortunately, TAH-BSO eliminates the disease and risk of recurrence in the majority of patients. However, despite the high success of treatment, as seen in the aforementioned survival rates, the experience often can affect patients’ quality of life and psychological well being (Klee & Machin, 2001).

The current results contribute to a growing body of literature suggesting that psychosocial factors contribute to biological processes known to influence cancer metastasis and progression (S. Lutgendorf, Anderson, Sorosky, Buller, & Lubaroff, 2000; S. K. Lutgendorf et al., 2005).
This relationship may also exist among healthy individuals. Indeed, HSP70 antibodies have been found in the circulation of healthy groups of people (Pockley et al., 1998; Rea, McNerlan, & Pockley, 2001) and similar HSP/anxiety relationships may be uncovered if anxiety were measured in healthy populations. Elevated circulating HSP60 was found in individuals with low socioeconomic status and, in women, with elevated psychological distress in a sample of healthy British Civil servants (Lewthwaite et al., 2002). Although the biological relevance of elevated HSP expression among healthy populations may not be well understood at this time, it may be an early indicator of systemic inflammation (Xu et al., 2000) and a marker of risk for a number of disease states (Ciocca & Calderwood, 2005; Herz et al., 2006; Pockley, Georgiades, Thulin, de Faire, & Frostegard, 2003).

The sample of participants presented here were primarily older ($M = 61.6$) and the majority were diagnosed with Stage 1 disease (47.4%). Moreover, this sample had elevated BMI’s ($M = 36.2$), with the majority of participants falling into the obese classification (67.6%), both of which are known risk factors for developing endometrial cancer (Trentham-Dietz, Nichols, Hampton, & Newcomb, 2006). Given that this sample was older in age, had less advanced stages of disease and presented with elevated BMI, the results can likely be generalized to other women with endometrial cancer. However, the subsample of participants who were analyzed here had significantly lower total years of education and total annual income compared to the larger sample from which they were selected. This suggests that the present findings may not be generalized to women of higher SES or education levels.

Women in this study endorsed low levels of psychosocial life stress, depressive and anxious symptoms. While there are no clinical cutoffs for the measures employed in this study, the means presented here fall in the lower third of each respective range of responses. In light of
these results, it is noteworthy that pre-operative anxious symptoms yielded a marginally significant relationship with HSP-antibodies. While the low levels of life stress may indicate that this sample of women were not experiencing high levels of distressing life experiences, it is noteworthy that many of the life experiences documented were from the “death of other relative” or “death of a close friend.” These life experiences may represent bereavement, which could potentially represent a unique life stressor and may impact clinical outcomes differently than other forms of life stress (Norris & Murrell, 1990).

In addition, it is striking that these relationships were demonstrated between HSP antibodies, given that the only human study examining psychosocial factors and HSPs investigated circulating levels of the protein (Lewthwaite et al., 2002) and not auto-antibodies. Measuring antibodies may represent a unique approach to the human body’s production of HSPs. For example, in the clinical literature, antibodies to HSPs have been related to breast cancer (Conroy, Sasieni, Fentiman, & Latchman, 1998), non-small cell lung cancer (Zhong et al., 2003), late-stage ovarian cancer (Luo et al., 2002), and is also associated with response to chemotherapy treatment (Trieb et al., 2000). Overall, both HSPs and their corresponding antibodies are related to the carcinogenic process suggesting that the exact physiological role of this class of proteins and their corresponding antibodies warrants further investigations.

**Study Limitations**

There are a number of limitations to the current study, and thus, the results should be interpreted with caution. Foremost of these limitations is the modest sample size, which limited statistical power. For instance, cortisol may have in fact mediated the relationship between anxiety and HSP70; however, the ability to detect this significant relationship may have been hampered by low statistical power. Another limitation is the low levels of anxious symptomatology in our sample of women. Yet another limitation is the cross-sectional design,
which precludes us from drawing any conclusions of cause and effect (that pre-operative anxiety causes elevated levels of HSP antibodies). In spite of these limits, important information was gained which may help direct future work in this area.

**Future Directions**

Future directions will include increasing the sample size of participants that collect full psychosocial and biological data as this will allow the mediational hypotheses presented here to be fully examined. Furthermore, examining relationships between HSP antibodies and additional mood states (e.g. clinical depression, other forms of life stress) may warrant further investigation in a larger sample not limited by statistical power.

As previously mentioned, performing an ELISA assay for HSP70 antigens (proteins) on blood samples from this same group of women will allow for a comparison between HSP70 antibody levels and circulating HSPs – further elucidating the role of these protective chaperone proteins. This additional assay would also allow for a more accurate comparison between the current results and other investigations of psychosocial factors and HSPs in human populations (Lewthwaite et al., 2002).

In addition, measuring HSP70 expression and anxiety within a group of female age-matched controls would allow the comparison of anxiety levels and HSPs in a group of individuals not undergoing surgery for suspected endometrial cancer. This would allow the opportunity to see if similar HSP70/anxiety relationships exist in healthy individuals – further clarifying if HSP70 expression is unique to endometrial cancer patients. Moreover, establishing a comparison for pre-operative anxiety levels would help clarify the level or degree of stress or anxiety measurable in the period prior to surgery for endometrial cancer. Given the limitation of only evaluating a single time point, it may prove advantageous to measure HSP over the course of patient’s treatment for endometrial cancer and include changes in psychological functioning.
with these future approaches. For example, the psychological stress leading up to the surgery date may dissipate after patients have recovered, and this could, in turn lead to reduced inflammatory markers and reduced levels of HSP70 antibodies.

Collectively, the present findings suggest that HSP70, a unique biological marker that is relevant in endometrial cancer progression and metastasis, is related to pre-operative anxiety. A number of investigators have suggested that HSPs may offer prognostic significance in endometrial cancer patients and are related to histological characteristics of tumors and disease course (Bai, Chen, Xin, & Wang, 2003; Li, Peng, & Yao, 1999; Nanbu et al., 1998). Drawing links between psychological factors and disease course may be premature at this point. However, it is interesting and potentially important that psychological factors such as stress and anxiety may indeed alter a circulating component such as HSP70 which reflect specific responses to carcinogenesis.


BIOGRAPHICAL SKETCH

Timothy Sannes graduated with honors from the University of North Carolina at Chapel Hill in 2004 with a Bachelor of Arts in psychology. Following graduation, Mr. Sannes worked as a Research Assistant at the University of North Carolina’s School of Medicine in the Department of Psychiatry. He then completed a Fellowship at the National Institute of Health in Complementary and Alternative Medicine investigating novel approaches to cancer treatment and recovery.

Timothy is majoring in clinical psychology at the University of Florida and received his M.S. in the spring of 2009. He is focusing his studies on psychoneuroimmunology and integrating his interest in alternative and mind-body therapies into these studies. He is currently pursuing his doctorate in clinical psychology.