Lehigh University

Annual Progress Report: 2011 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Formula Grant Overview

Lehigh University received $80,151 in formula funds for the grant award period January 1, 2012 through June 30, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Automated Analysis of Microtubule Dynamics to Study Cytoskeleton-Targeted Chemotherapies – The purposes of this project are to determine whether the tumor suppressor protein, p53, regulates the dynamic turnover of the microtubule cytoskeleton and whether p53 expression protects cells from the chemotherapeutic drug, Taxol. Automated computer-based image analysis methods will be applied throughout.

Duration of Project

1/1/2012 – 6/30/2014

Project Overview

The gene for p53, encoding a critical tumor suppressor protein, is mutated in at least 50% of all human cancers and lack of p53 is thought to facilitate cancer cell survival and tumor progression. Several functions have been ascribed to p53 in addition to its role as a transcription factor, including regulating microtubule assembly dynamics. This latter function is of interest because cells harboring mutations in, or loss of, p53 are most susceptible to Taxol, a chemotherapeutic drug that prevents microtubule disassembly. While we know much about how Taxol stabilizes microtubules, we still do not know how Taxol treatment ultimately causes cell death and why Taxol appears to be much more effective in cells lacking p53.

Specific Aims:
Aim 1: Adapt automated particle tracking methods developed by others. Two research groups have developed automated tracking methods to detect the position of all GFP- EB1-marked microtubule ends within an image. Each group built their analysis system in Matlab and each group freely shares their software with the research community. Our first goal is to optimize image acquisition conditions to best suit computer-based image recognition methods and to determine which algorithm provides the best analysis tools for both interphase and mitotic cells.
Aim 2: Does p53 regulate microtubule dynamics and antagonize Taxol? Our major goal is to examine microtubule assembly in cells manipulated to vary p53 expression and to examine how

the cell's response to Taxol (at the level of microtubule assembly) depends on its p53 status. We will use transient expression of EB1-GFP in several cell lines and manipulate p53 as we have described previously.

Principal Investigator

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Other Participating Researchers

Daniel Ciccone – employed by Lehigh University
Cody Molnar, graduate student, Lehigh University

Expected Research Outcomes and Benefits

Completion of the proposed research will possibly describe a new function for p53, the most frequently mutated gene in human cancers and a mutated gene most responsible for cancer progression. We expect that p53 will contribute to microtubule assembly and turnover within cells and make them resistant to Taxol, a chemotherapeutic drug used to treat breast, ovarian and prostate cancer, among others. Understanding why certain cancers are susceptible to Taxol, while others are not, could lead to ways to increase Taxol's efficacy.

Summary of Research Completed

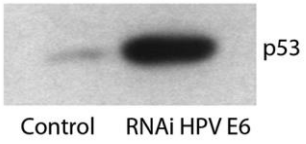
The past year's efforts were focused on Aim 2 of the proposal. That Aim is described here:
Aim 2: Does p53 regulate microtubule dynamics and antagonize Taxol? Our major goal is to examine microtubule assembly in cells manipulated to vary p53 expression and to examine how the cell's response to Taxol (at the level of microtubule assembly) depends on its p53 status. We will use transient expression of EB1-GFP in several cell lines and manipulate p53 as we have described previously.

We confirmed our ability to manipulate p53 level in Hela cells by knockdown of the E6 protein. This protein, encoded by the HPV genome, normally targets p53 for destruction. See Figure 1 for an example of p53 restoration by E6 knockdown.

We then used our automated image analysis system to ask (a) whether p53 level influences microtubule assembly dynamics and (b) whether p53 level influences microtubule response to Taxol. In both cases we found that microtubule dynamics and their stabilization by Taxol were unaffected by p53 level. Figure 2 shows the major findings in graph format. Importantly, our analyses included data from over 14,000 microtubules for each experimental condition. Given the stochastic switches between growth and shortening characteristic of microtubule dynamic

instability, these large sample sizes ensure statistically significant values.

Figure 1. Immunoblot demonstrating manipulation of p53 protein level. P53 is restored in Hela cells by depleting them of the HPV viral protein E6. Normally in these cells, E6 targets p53 for destruction. Depleting cells of E6 allows p53 to return to a level typical of epithelial cells. These conditions were used to probe p53-dependent changes to the microtubule cytoskeleton.



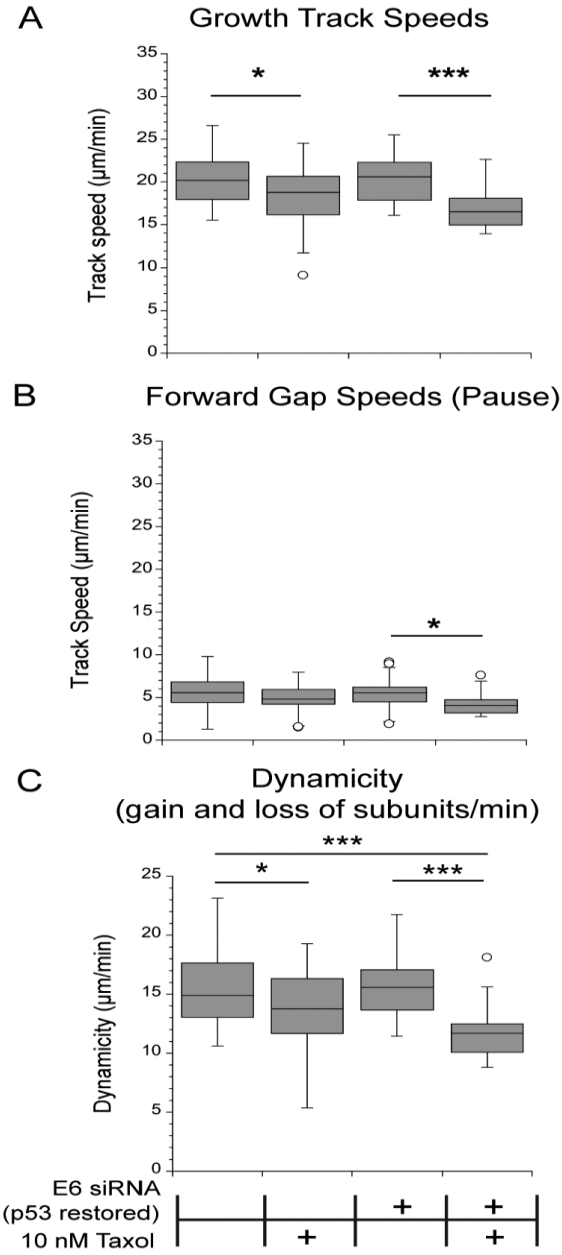


Figure 2: Microtubule dynamics in response to p53 level and/or 10 nM Taxol. Cells were incubated in 10 nM Taxol for 4 hours prior to imaging. Dynamicity is a sum of the gain and loss of tubulin subunits per unit time and is a convenient measure for comparison between treatments. Note that microtubule polymers contain 1624 tubulin dimers/µm, so dynamicity values of 15 µm/min represent the gain and loss of tens of thousands of subunits each minute. Addition of Taxol slows microtubule growth and reduces dynamicity independent of p53 status. Values shown here represent data from the entire cell volume.

Research Project 2: Project Title and Purpose

Cortisol, Estradiol, and Psychosocial Stress as Predictors of Postpartum Depressive Symptoms – The purpose of this project is to examine how prenatal psychosocial stress and the hormones cortisol and estradiol interact to affect risk for postpartum depression (PPD). Research shows that prenatal stress can increase risk for PPD. The body's reaction to stress is regulated by cortisol, and elevated levels of estradiol can inhibit cortisol's ability to do so. Both cortisol and estradiol increase dramatically during pregnancy, which may increase sensitivity to stressful events. This project uses twice-daily saliva samples and brief questionnaires to examine bidirectional associations between these hormones and reports of stress during a one-week period during pregnancy, as well as how their connections predict postpartum depressive symptoms.

Duration of Project

1/1/2012 – 6/30/2014

Project Overview

Hormonal changes are often cited as key triggers of postpartum depression (PPD). However, mechanisms may be difficult to pinpoint because the links between hormonal changes and psychosocial events can be bidirectional. This bidirectionality may be especially important in the case of PPD, as pregnancy and the postpartum are marked by both hormonal changes and the experience of stressful events (which have also been linked to risk for PPD). This project focuses on the roles of two specific hormones relevant to the stress-PPD link. Cortisol (CORT) serves the important function of countering the activation of the hypothalamus-pituitary-adrenal (HPA) axis, which is activated in response to stress. Estradiol (E2) inhibits the ability for CORT to perform this necessary function, suggesting an interaction between CORT and E2 predicting the intensity and duration of stress reactions. Basal concentrations of both hormones increase throughout pregnancy, and the diurnal cycle of CORT is dampened. The aims of this project are:

1) Examine the day-to-day links between CORT, E2, and stress during pregnancy. Namely, (a) daily stress should increase evening CORT, particularly when E2 is also elevated; (b) elevated evening CORT should predict poor sleep and physical symptoms on waking; and (c) poor sleep and physical symptoms on waking should increase susceptibility to stress later in the day.

2) Probe the associations between this daily process and PPD risk. Higher levels of (and greater increases in) CORT indicate higher levels of stress, which should predict depressive symptoms in the postpartum (adjusting for risk factors like history of depression). This link may be especially strong for women with higher levels of (or more rapidly increasing) levels of E2.

This project is a three-phase longitudinal study of pregnant women. In the first phase (~27 weeks gestation), women will report baseline depressive symptoms, history of mental illness, perceived stress, and recent life events. The second phase (~31 weeks gestation) involves completing brief questionnaires about daily experiences and providing saliva samples for hormone assay twice daily (upon waking, and just before bed) for one week. Finally, a postpartum questionnaire (~4 weeks postpartum) will reassess depressive symptoms and other relevant variables.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

This project will result in a rich set of data describing the natural progression of stress and depressive symptoms during pregnancy. The combination of twice-daily self-report questionnaires and hormone assays will provide unusually high-resolution information on the complex interconnections between psychological and physiological indicators of stress during pregnancy. Consequently, this project should result in novel insights regarding how these two levels of analysis interact to predict depressive symptomatology in the postpartum period. In addition, the longitudinal nature of the study will allow for the examination of individual differences in (a) the links between hormones and subjective experiences of stress and (b) the links between stress and depressive symptoms. Some of these individual differences may be accounted for by socio-demographic factors (e.g., socioeconomic status), while others may be explained by dispositional factors (e.g., emotion regulation ability, coping skills). Thus, at the broadest level, this project should advance understanding of the complex etiology of postpartum depression.

Summary of Research Completed

During the period 7/1/2013 to 6/30/2014, research activities focused initially on recruitment and data collection. We continued advertising for the study in local restaurants, and in February we enrolled our final participant. Table 1 provides recruitment totals, both overall and for the current period. During this period, 7 of 15 (47%) women who contacted us were eligible and agreed to participate, in line with the overall trend (42 of 106, or 40%). One participant dropped out prior to completion of the prenatal phase during this period (over the entire study period, 3 of 42, or 7% did so). Aside from this one dropout, all participants completed the postpartum phase during this reporting period. Over the entire study, 4 of 39 (10%) of participants who completed the prenatal phase dropped out prior to completing the postpartum phase. Table 1 also summarizes our data collection efforts during this reporting period. We sent two batches of saliva samples to the testing services company to be assayed for cortisol and estradiol. The first batch contained samples from 14 participants (193 samples), and the second contained samples from 13 participants (182 samples). All remaining participants also completed the survey components.

With all of the data in hand, I compiled the data sets from the various sources, and cleaned the data as necessary. Data cleaning primarily involved verifying that date/time stamps on online surveys fell within an acceptable window of time and checking for incorrect or out of range responses. For instance, in one case a participant entered the wrong identification number, which was corrected. Out of range responses that could not be reconciled were recoded as missing. A special note should be made regarding the "acceptable window of time" for completing surveys and saliva samples. Participants were instructed to complete the morning and evening surveys and saliva samples within 60 minutes of getting out of bed and within 60 minutes of going to bed, respectively. As a first pass, I excluded morning responses completed more than two hours after their self-reported waking time, and for the evening survey I excluded responses completed outside of the window 6:00pm-6:00am. However, given the presence of diurnal cycles in the hormones of interest, I wanted to ensure that any surveys/samples completed outside of the appropriate times in the daily sleep-wake cycle were removed from the data. With the remaining cases, I sorted the data by completion time, identified extreme values, and cross-checked each with the time stamps for (a) the corresponding survey (i.e., morning or evening) on different days and (b) the previous and subsequent surveys for that subject. Responses were excluded if completion times were inconsistent with the subject's general pattern of responding and if the survey was completed close in time to an adjacent survey, indicating that subjects did not complete them at the appropriate time in their daily sleep-wake cycle. Finally, I eliminated the data from one subject who reported that she sometimes worked a night shift, as it became unclear which surveys/samples to treat as "morning" and which to treat as "evening". These data exclusion rules were formulated and applied prior to exploratory data analysis. Below, I report the preliminary results of statistical analyses designed to test the Specific Aims of the project.

Specific Aim 1: Examine the day-to-day links between CORT, E2, and stress during pregnancy. Namely, (a) daily stress should increase evening CORT, particularly when E2 is also elevated; (b) elevated evening CORT should predict poor sleep and physical symptoms on waking; and (c) poor sleep and physical symptoms on waking should increase susceptibility to stress later in the day.

Specific Aim 1 contains a set of three hypotheses concerning the carrying forward of stress experiences over time. For clarity of presentation, I will report the results in a slightly different order than originally listed. Specifically, I first tested how evening CORT, E2, and distress predict sleep quality, physical symptoms, and distress the following morning. I then tested how these morning factors predicted stress experiences later in the day, and whether these effects were modified by morning CORT and E2. Third, I report analyses testing how daily stress impacts evening distress, CORT and E2, and whether these effects were modified by morning distress, CORT, and E2.

I used mixed (or multilevel) model analyses to test each of these questions due to the nested structure of the data (observations nested within person). All within-subjects (i.e., daily) predictors were person-centered, such that fixed effect estimates can be interpreted as the expected change in the outcome for each unit deviation from the individual's mean on the predictor. To ensure that individual differences in predictors were not lost, the between-person means were included in the models as well (after being centered at their respective grand means).

Do evening CORT, E2, and distress predict sleep quality, morning physical symptoms, and morning distress?

Throughout the analyses, sleep quality, morning physical symptoms, and number of night wakings exhibited a very similar pattern, and they showed moderate positive correlations with each other (*r*s ranged from 0.42 to 0.66). Therefore, for simplicity of presentation, I standardized each and averaged these standardized scores to form an index of sleep quality, which had a reliability of approximately 0.72. This first analysis revealed, contrary to expectations, that evening CORT, E2, and distress did not predict the sleep quality index (ts < 0.88, ps > .378). However, effects did emerge in the analysis predicting morning levels of distress. In particular, there was a significant two-way interaction between evening CORT and evening E2 predicting morning distress ($t(114) = 2.23$, $p = .028$; see Figure 1). A simple slopes analysis revealed that when evening E2 was 1 standard deviation (SD) higher than average, there was no association between evening CORT and morning distress ($t(114) = 1.00$, $p = .319$). When evening E2 was 1 SD below average, there was a significant negative association between evening CORT and morning distress, $t(114) = 2.08$, $p = .040$. As seen in Figure 1, this pattern suggests that when E2 is high in the evening, morning distress is equally high regardless of evening CORT. However, when E2 is low in the evening, higher evening CORT actually predicts less distress in the evening. This analysis also revealed that individuals with higher evening CORT on average reported significantly higher morning distress, $t(114) = 2.51$, $p = .014$. Thus, although the predicted effects were not apparent for the sleep-related variables, this analysis provides some support that elevated evening CORT and E2 carry forward to morning distress.

Do sleep quality and morning mood predict higher levels of stress later in the day, and do these effects depend on morning CORT and E2?

The next step in the analysis was to examine whether sleep quality and distress would in turn influence the experience of stress later in the day. There were three ways in which daily stress was assessed in the evening survey. The first was a rating of the overall stressfulness of the day (measured on a scale from 1 to 5). Participants also briefly described the most stressful event or aspect of the day. Their rating of the stressfulness of that event (measured on the same scale as overall stressfulness) constituted the second index of daily stress. The third was a count of common troublesome events that participants selected from a checklist of fifteen such events used in past research (e.g., extra work at home, back/muscle ache, negative feedback or evaluation). These measures were substantially positively correlated (*r*s ranged from .36 to .70). Considering them as a set, they had a reliability of approximately 0.70. Given this result, for simplicity of presentation, I standardized each of the variables and averaged them together to create a daily stress index. The results reported below use this index as the outcome variable.

Paralleling the findings from the previous set of analyses, the sleep quality index did not predict daily stress, either alone or interacting with morning CORT and E2. Once again, the analysis using morning distress in place of the sleep quality index provided results more in line with the hypotheses. There was a significant main effect of morning distress on reports of stress ($t(130) = 2.73$, $p = .007$), such that higher morning distress predicted having a more stressful day. This effect was qualified by a significant interaction between morning distress and morning CORT, $t(130) = 3.09$, $p = .002$. There was a significant main effect of morning E2 ($t(130) = 2.23$, $p =$

.027), such that higher morning E2 predicted greater stress experienced during the day. This effect of E2 was significantly stronger to the extent that morning CORT was also elevated, $t(130) = 3.18$, $p = .002$. Finally, there was a significant three-way interaction between morning distress, E2, and CORT predicting daily stress, $t(130) = 1.92$, $p = .058$. As seen in Figure 2, there was only a significant positive association between morning distress and reports of stress experienced later in the day when both CORT and E2 were higher than average in the morning, $t(130) = 2.34$, $p = .021$. The link between morning stress and daily stress was nonsignificant when morning E2 was low and CORT was high ($t(130) = 1.35$, $p = .181$) and when CORT was low and E2 was high ($t(130) = 1.38$, $p = .169$). When both morning E2 and morning CORT were below average, there was a marginal positive association between morning distress and later reports of stress, $t(130) = 1.92$, $p = .057$. Thus, this analysis generally supported the prediction that higher morning distress would predict experiencing more stress later in the day, particularly when CORT and E2 were also elevated.

<u>How does stress experienced during the day relate to evening distress, CORT, and E2, and do these effects depend on morning distress, CORT, and E2?</u>

The final set of analyses for Specific Aim 1 examined how morning distress, CORT, and E2 modified the impact of daily stress on evening distress, CORT, and E2. Once again, I used the composite index of daily stress. The stress composite variable did not predict evening CORT or E2, either directly or interacting with morning CORT and E2 (ts < 1.53, ps > .130). The stress composite did, however, predict an increase in evening distress, $t(130) = 5.66$, $p < .001$. This effect was somewhat weaker to the extent that morning distress was elevated ($t(130) = 2.09$, $p = .039$), perhaps because morning anxiety was elevated in anticipation of daily stressors. There was a significant two-way interaction between morning distress and morning E2 ($t(130) = 2.96$, $p = .004$), which was qualified by a three-way interaction between morning distress, E2, and daily stress, $t(130) = 5.10$, $p < .001$. The pattern of this effect (see Figure 3) somewhat paralleled the pattern in Figure 2: When both morning distress and morning E2 were high, there was a significant positive association between daily stress and evening distress, $t(130) = 4.78$, $p < .001$. A similar effect emerged when both morning distress and E2 were low, $t(130) = 6.16$, $p < .001$. When morning distress was high and E2 low (and vice versa), the effect of daily stress on evening distress did not reach significance, ts < 1.70, ps > .093.

Thus, the set of analyses associated with Specific Aim 1 provided some evidence of the hypothesized process. Namely, evening levels of CORT and E2 predicted elevated distress in the morning, which in turn predicted the degree of stress experienced by the individual later in the day. The carrying forward of morning distress was most pronounced when both CORT and E2 were also elevated in the morning. Finally, daily stress predicted the highest level of evening distress when both morning distress and E2 were higher than average. This elevated evening distress should then carry forward to the next morning, continuing the cycle.

Specific Aim 2: Probe the associations between this daily process and PPD risk. Higher levels of (and greater increases in) CORT indicate higher levels of stress, which should predict depressive symptoms in the postpartum (adjusting for risk factors like history of depression). This link may be especially strong for women with higher levels of (or more rapidly increasing) levels of E2.

To test the second specific aim, I conducted a series of linear growth curve analyses predicting each variable (morning and evening distress, CORT, and E2) by study day, adjusting for day of the week (because each might vary systematically between, for instance, workweek and weekend). I allowed for between-subject variable in both the intercept and slope. I then predicted postpartum depressive symptoms from this intercept, slope, and their interaction. In this analysis, I adjusted for prenatal depressive symptoms to rule out the possibility that it was pre-existing depression rather than these daily experiences variables that were driving the elevated postpartum depressive symptoms. Only one of these analyses resulted in a positive finding, so I report only that one. I found that there was significant variability in the intercept of morning distress (SD = 0.31 on the 1-5 scale of morning distress). Because the slope of morning distress was not significant, this intercept variability can be thought of as a basic individual difference in morning distress. This variability in morning distress, in turn, predicted higher levels of depressive symptoms postpartum, adjusting for prenatal depressive symptoms, $t(30) = 3.28$, $p = .003$. Each standard deviation increase in initial morning distress was associated with a 2.8-point (or 0.68-SD) increase in postpartum depressive symptoms. Although I was hoping to find links between the slopes and postpartum depressive symptoms, this analysis provides some preliminary support for the connection between the daily stress process during pregnancy and postpartum depressive symptoms.

The final purchases I made during this reporting period will help as I translate these findings into publications and additional grant proposals. I purchased Mplus software, a cutting edge statistical package, to refine the growth curve analyses associated with Specific Aim 2. I also purchased several books, including recently published books on advanced statistical methods, stress and health, and psychoneuroendocrinology. These books will be highly valuable as I write up these results for publication. They will also help me understand some of the unexpected data patterns, which will aid in refining my hypotheses when applying for future funding.

Table 1. Recruitment and data collection overall and during the period 7/1/2013-6/30/2014

	Overall	Reporting period 7/1/2013-6/30/2014
Initial contact	106	15
Agreement to participate	42	7
Commence prenatal phase	42	16
Complete prenatal phase	39	16
Commence postpartum phase	35	23
Complete postpartum phase	35	23
Saliva samples collected	39	18
Saliva samples processed	39	27

Figure 1. Interaction of last evening's CORT and E2 predicting morning distress

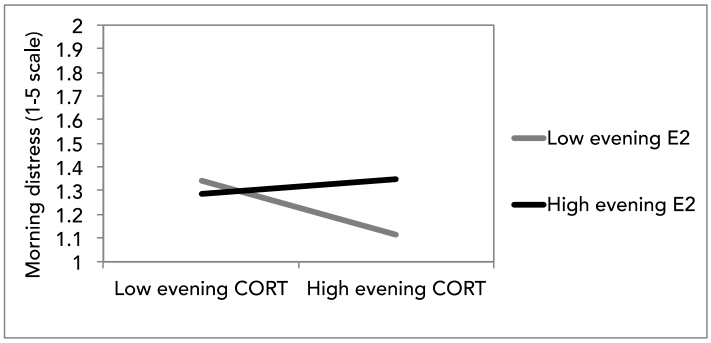

Figure 2. Interaction of morning distress, CORT, and E2 predicting stress composite index

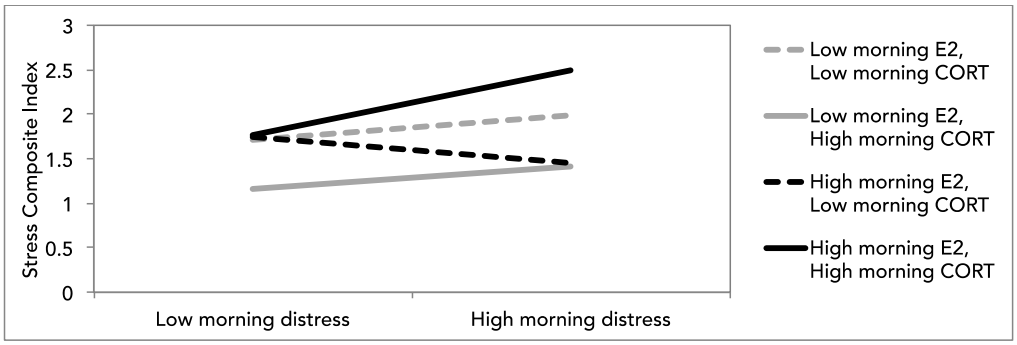
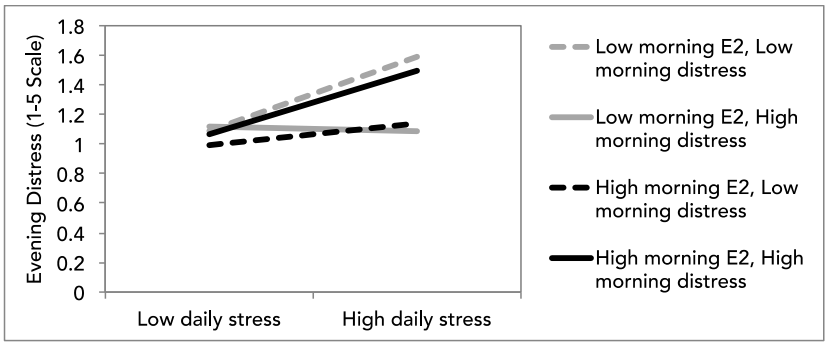
Figure 3. Interaction of daily stress, morning distress, and E2 predicting evening distress