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# Assessing Correlations Between Cortisol and Symptoms of Depression in Idaho Resident Freshmen

by

Emily Baergen

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in the Department of Medical Laboratory Science

Idaho State University

Fall 2019

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Emily Baergen find it satisfactory and recommend that it be accepted.

_____

Rachel Hulse,

Major Advisor

_____

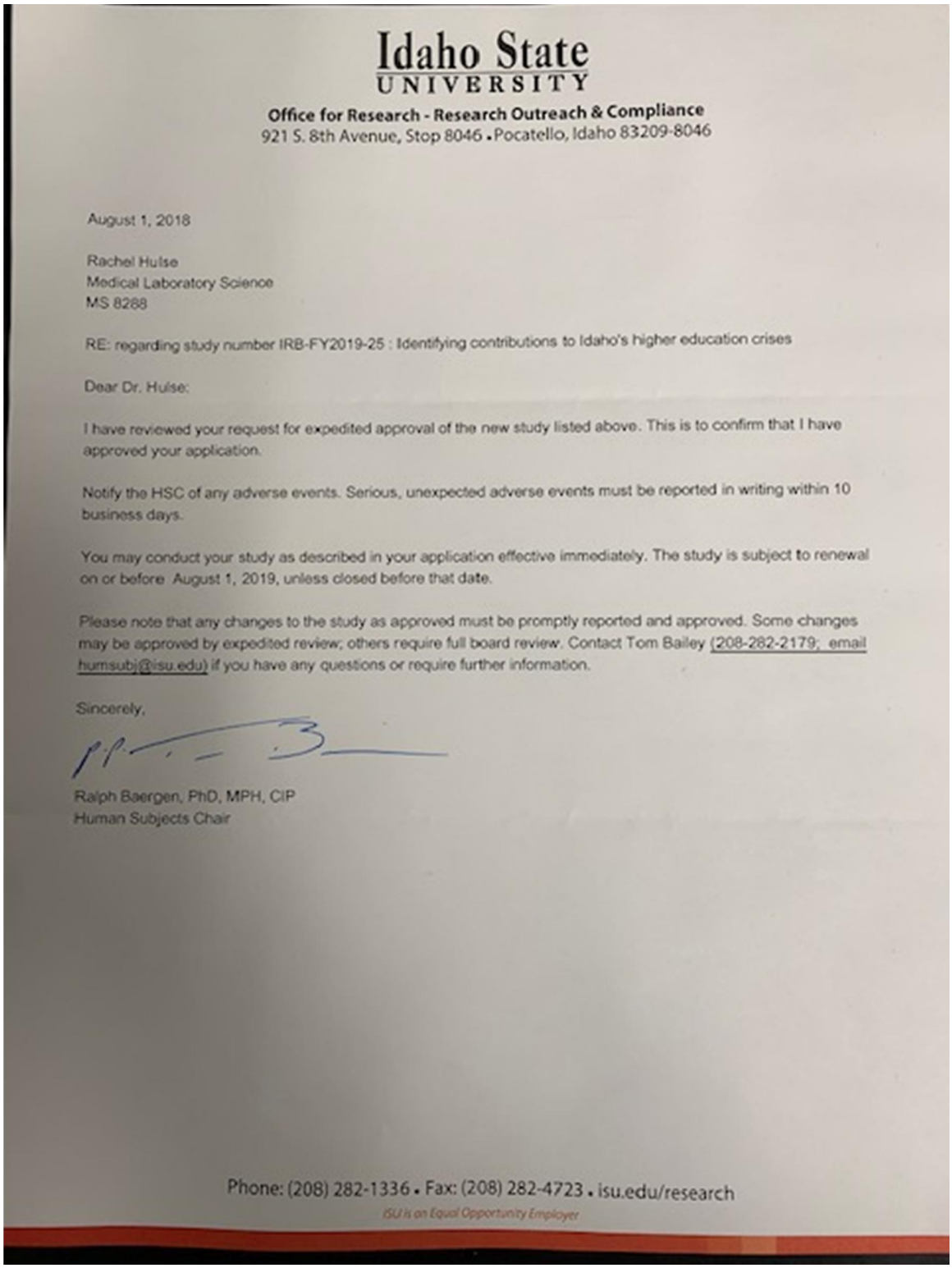
Marjorie Montanus,

Committee Member

_____

Ryan Lindsey,

Graduate Faculty Representative



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UNIVERSITY

Office for Research - Research Outreach & Compliance
921 S. 8th Avenue, Stop 8046 • Pocatello, Idaho 83209-8046

August 1, 2018

Rachel Hulse
Medical Laboratory Science
MS 8288

RE: regarding study number IRB-FY2019-25 : Identifying contributions to Idaho's higher education crises

Dear Dr. Hulse:

I have reviewed your request for expedited approval of the new study listed above. This is to confirm that I have approved your application.

Notify the HSC of any adverse events. Serious, unexpected adverse events must be reported in writing within 10 business days.

You may conduct your study as described in your application effective immediately. The study is subject to renewal on or before August 1, 2019, unless closed before that date.

Please note that any changes to the study as approved must be promptly reported and approved. Some changes may be approved by expedited review; others require full board review. Contact Tom Bailey (208-282-2179; email humsubj@isu.edu) if you have any questions or require further information.

Sincerely,

Ralph Baergen, PhD, MPH, CIP
Human Subjects Chair

Phone: (208) 282-1336 • Fax: (208) 282-4723 • isu.edu/research
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TABLE OF CONTENTS

# List of Figures

# List of Tables

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACTH | Adrenocorticotropin hormone |
| AUC | Area under the curve |
| BAI | Beck anxiety inventory |
| BDI-II | Beck Depression Inventory-II |
| CAR | Cortisol awakening response |
| CES-D | Center for Epidemiological Studies depression scale |
| CRH | Corticotropin-releasing hormone |
| DASS | Depression, anxiety, and stress scale |
| ECT | Electroconvulsive therapy |
| ELISA | Enzyme-linked immunosorbent assay |
| GHQ | General health questionnaire |
| HAM-D | Hamilton depression rating scale |
| HPA | Hypothalamic-pituitary-adrenal |
| ISU | Idaho State University |
| LC-MS | Liquid chromatography-mass spectrometry |
| MDD | Major depressive disorder |
| MDI | Medical depression inventory |
| PHQ-9 | Patient health questionnaire – 9 items |
| PSS | Cohen’s perceived stress scale |
| SSRI | Selective serotonin reuptake inhibitor |
| TCA | Tricyclic antidepressant |

Assessing Correlations Between Cortisol and Symptoms of Depression in Idaho Resident Freshmen

Thesis Abstract – Idaho State University (2019)

College freshmen experience unique stressors when transitioning from high school to college. In Idaho, the freshman attrition rate is higher than the national average, which may be partially explained by the higher prevalence of depression in this region. The current study investigated the potential of cortisol, a stress hormone, to be a biomarker for depression to help identify those freshmen with depression that may be at a higher risk of dropping out. In a group of 45 college freshmen, no correlations were observed between serum cortisol and depression severity as measured by the Patient Health Questionnaire 9 (PHQ-9). However, those with a self-reported history of depression scored significantly higher on the PHQ-9, confirming the reliability and validity of the PHQ-9 in a college student population.

Keywords: PHQ, serum cortisol, freshman, attrition, Idaho, depression

# Chapter I: Introduction

Higher education in Idaho is struggling. Idaho ranks last in the nation in the proportion of high schoolers that go on immediately to college after graduating from high school. In addition, only 70.4% of Idaho college freshmen return for their sophomore year, which corresponds to a rank of 43rd in the nation.

Some of the risk factors for increased attrition are being an ethnic minority, low socioeconomic status, disability, and mental illness (O'Keefe, 2013). Due to the higher incidence of high depression and suicide rates in the Mountain West region, mental health in college freshmen and its effects on attrition rates in this area of the country is a relationship that is worth further exploration (Watson, 2019, University of Washington Institute for Health Metrics and Evaluation, 2019).

Improving mental health services on college campuses is the natural next step in reducing attrition rates among college students, but students requiring mental health services must first be identified. Currently, there is no biomarker that has been identified for depression. Research has been done on the potential for cortisol to be a biomarker for depression. Cortisol is a stress hormone that is released in response to psychological distress (Burtis and Bruns, 2015).

Research question: Is cortisol concentration an effective biomarker for depression that could be utilized as a screening tool to identify college freshmen in Idaho that are suffering from depression?

Hypothesis: High serum cortisol concentrations will correlate with depression severity in Idaho college freshmen.

To test our hypothesis, we recruited 45 Idaho State University (ISU) college freshmen who were also Idaho residents. We measured their serum cortisol concentrations in the morning and evening on one day at the beginning of their first semester of college and again at the end of their first semester. At the same time as each cortisol sampling, subjects completed the Patient Health Questionnaire 9 (PHQ-9), a brief screening tool that assesses whether the subject is depressed, and if the subject is depressed measures the severity of the depression (Spitzer, Kroenke, and Williams, 1999). Subjects also completed a brief survey of demographic information and history of mental health. We then examined the results for any correlations between PHQ-9 score and cortisol concentration.

# Chapter II: Literature Review

## Challenges for College Freshmen

Beginning college represents a major life change; many students move away from home and must adjust to new stressors, both social and academic. This also represents a time when the student must take on more adult responsibilities (Pedrelli et al., 2015). With this unique set of stressors during this time of transition, freshmen in particular experience high rates of attrition in comparison to the other undergraduate class levels (Pedrelli et al., 2015).

## Freshmen in Idaho

Idaho is currently in the midst of a higher education crisis. In Idaho, only 41% of adults aged 25 to 34 have a 1-year certificate or more. The state of Idaho has set a goal to increase this number to 60% by the year 2020 (Idaho State Board of Education, 2012). In 2017, only 48% of Idaho high school seniors continued their education at college in the fall immediately following high school graduation (ISBOE, 2018). This is a rank of 50$^{th}$ in the nation (National Center for Higher Education Management Systems, 2014). Only 70.4% of those that completed the first year of a full-time course load returned to college for the second year, which corresponds to a rank of 43$^{th}$ in the nation in 2015 (NCHEMS, 2015).

At Idaho State University (ISU), freshman retention is even lower. In 2018, only 65% of freshmen returned to ISU for their second year of college (ISBOE 2018).

## Depression and Stress

Depression is characterized by symptoms such as loss of interest in activities, sad or "empty" mood, lack of energy, sleep problems (excess sleeping or insomnia), pessimism, and physical pain. Among U.S. adults, 7.1%, or 17.3 million adults, experienced a major depressive episode in the preceding 12 months. The prevalence of depression in adults is highest in the 18-25 year age group, where it is 13.1% (National Institute of Mental Health, 2019).

**Depression in higher education.** Major life changes and stress are both risk factors for depression (National Institute of Mental Health, 2018). Beginning college often presents these risk factors, and many students either experience depression for the first time or experience a relapse of existing depression (Pedrelli et al., 2015). A review by Ibrahim, Kelly, Adams, and Glazebrook (2013) found that university students in the United Kingdom experienced a higher prevalence of depression than the general population. In a study of college students at an Ohio university, Beiter et al. (2015) found that 11% of students showed severe or extremely severe levels of depression as measured by the 21-question version of the Depression, Anxiety, and Stress Scale (DASS).

According to a report published in 2019 by the American College Health Association, 19.9% of college undergraduates surveyed reported that they had been diagnosed with and/or treated for depression in the previous 12 months, and 24.9% reported that they had been diagnosed with depression in the past. In addition, 11.4% of undergrads reported that their depression had resulted in a lower grade or an "incomplete" in a course, dropping a course, or experiencing a significant disruption in their thesis. This report surveyed 54,497 students at 98 schools across the United States.

Eisenberg, Hunt, and Speer (2013) surveyed 14,175 students from 26 different colleges. They found that 17.3% of the students had a positive screen for depression; 9% of these scored in the major depression range, and 8% scored in the less severe range. A follow-up study recently found that the rate of depression in college students has increased. Lipson, Lattie, and Eisenberg (2019) found that students during the 2016-2017 school year showed a 29.9% prevalence of depression as measured by the PHQ-2.

In a study of Belgian college freshmen, Bruffaerts et al. (2018) found that 34.9% had experienced a mental health problem in the previous 12 months. These mental health problems were accompanied by a decrease in grade point average (GPA) of 0.2 to 0.3.

**Biomarkers of Depression and Stress**

Cortisol is a hormone that is secreted by the adrenal cortex. It is part of the hypothalamic-pituitary-adrenal (HPA) axis. A specific stimulus, such as physical or psychological stress, exercise, or hypoglycemia, triggers the secretion of corticotropin-releasing hormone (CRH) from the paraventricular cells of the hypothalamus. This stimulates the anterior pituitary gland to secrete adrenocorticotropin (ACTH), which stimulates the adrenal cortex to synthesize and release cortisol. The HPA axis is controlled by a negative feedback system; cortisol suppresses CHR secretion from the hypothalamus and suppresses ACTH secretion from the anterior pituitary (Burtis and Bruns, 2015). Figure 2.1 illustrates the HPA axis. Cortisol secretion shows a diurnal pattern of release. It is high upon waking, reaches a peak approximately 30 minutes after waking, and then falls

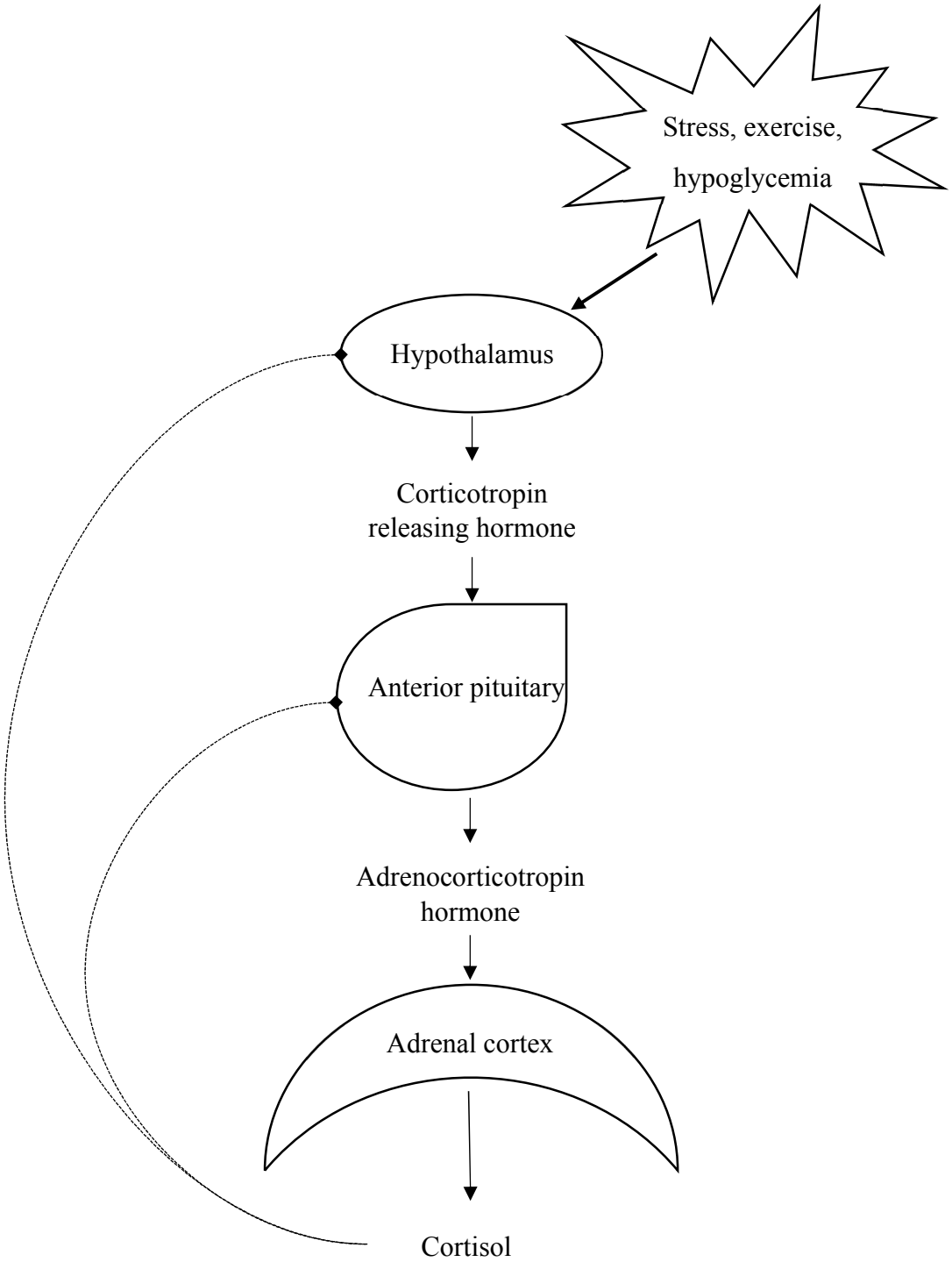


*Figure 2.1.* Hypothalamic-pituitary-adrenal (HPA) axis schematic.

through the day to reach its lowest point at night (Adam, 2017). Figure 2.2 shows an example of a normal cortisol diurnal concentration curve.

Cortisol can be measured in subjects' plasma and serum, which requires a blood draw. More commonly, however, it is measured in subjects' saliva because of ease of obtainment and its low invasiveness. To obtain a saliva sample for cortisol analysis, subjects simply must chew on a piece of cotton or passively drool into a sample container. However, although saliva cortisol levels are highly correlated with serum cortisol levels (Estrada-Y-Martin et al., 2011), saliva cortisol levels show much more variability than plasma or serum cortisol levels (Hoifodt et al., 2019, Dorn et al 2007).

Another consideration when evaluating cortisol levels is the number of samples taken and more importantly the timing of sampling throughout the day since cortisol levels are highly rhythmic. Many studies utilize samples taken at least twice throughout the day and select a morning and evening timepoints, which typically represent the approximate maximum and minimum cortisol levels, respectively (Yonekura, Takeda, Shetty, and Yamaguch, 2014, Hoifodt et al., 2019, Hinkelmann et al., 2009, Krogh et al., 2012). Another consideration in measuring cortisol is whether the timepoints will be a set times throughout the day or based on the individual's sleep pattern (i.e., cortisol sampling is timed to when the subject wakes in the morning and goes to sleep at night).

Many researchers choose to sample cortisol levels more frequently than twice a day, in particular sampling more frequently in the morning so that the cortisol awakening response (CAR) may be assessed (Doolin et al, 2017, Vreeburg et al., 2009, Manthey et

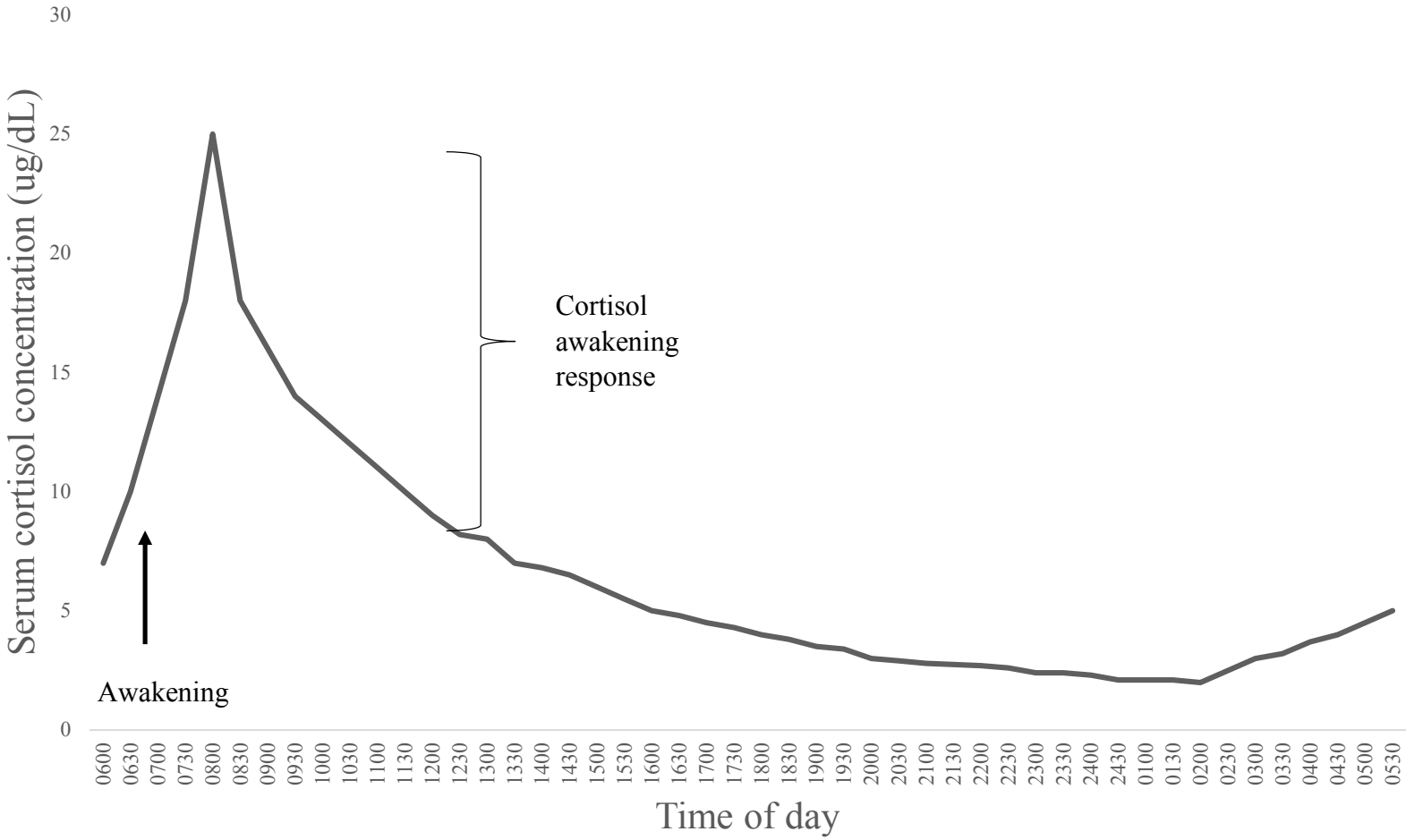


*Figure 2.2.* Example of a healthy cortisol concentration curve over 24 hours. In this example, the subject awakens at approximately 0700 h. Cortisol begins to rise a few hours before awakening and then peaks in concentration 30 to 60 minutes after awakening. Cortisol concentration then falls through the day to reach a nadir in the early morning hours (0200 h in this example).

al., 2011). The CAR includes the cortisol level immediately upon waking and also the levels 30 to 60 minutes following waking (see Figure 2.2).

Yonekura, Takeda, Shetty, and Yamaguchi (2014) looked at the correlation between salivary cortisol and depression in adolescents (mean age of 14 years) following the Tohoku Earthquake in Japan. They measured depression severity in 63 subjects over three days using the General Health Questionnaire-28 (GHQ-28). They also measured salivary cortisol levels at three set times each day over three days. Cortisol was measured using enzyme-linked immunosorbent assay (ELISA). The "high symptom" group (the 14 subjects scoring the highest on the GHQ-28) had a significantly higher average evening cortisol level than the "low symptom" group (the remaining 49 subjects with the lower GHQ-28 scores). The "high symptom" group also had a significantly higher slope (morning cortisol level divided by evening cortisol level) than that of the "low symptom" group.

Tailoring sampling times to the subjects' individual sleep patterns, Hoifodt et al. (2019) compared salivary cortisol levels upon wakening and in the evening (between 8 p.m. and 1 a.m.) in currently depressed (n=37), previously depressed (n=81), and non-depressed (n=50) subjects. Depression was assessed using a clinical interview and the Beck Depression Inventory-II (BDI-II). Cortisol was measured using an electrochemiluminescence immunoassay. There was a significant positive correlation between evening cortisol and BDI-II scores. In addition, the currently depressed group had a significantly higher evening cortisol level when compared to the never depressed group.

Doolin et al. (2017) compared salivary cortisol levels using liquid chromatography-mass spectrometry (LC-MS), a highly-sensitive method. They measured cortisol at five timepoints throughout the day in subjects with and without major depressive disorder (MDD): immediately upon waking, and then 30, 60, 720, and 750 minutes after waking. They also assessed current depression symptoms in all subjects using the Hamilton Depression rating scale (HAM-D) and the Center for Epidemiological Studies-Depression scale (CES-D), finding that those with MDD scored significantly higher on both scales. Salivary cortisol in subjects with MDD was significantly higher than control subjects immediately upon waking in the morning. The CAR was also perturbed in depressed patients. In those with MDD, a regression line fit through the 3 morning timepoints had a lower slope than that of the controls. Further, among those with MDD, HAM-D scores correlated with average morning cortisol concentration and peak morning cortisol concentrations.

Hinkelmann et al (2009) examined salivary cortisol between subjects with major depressive disorder (n=52) and healthy controls (n=50) at four set time points throughout the day. The cortisol sampling times were not based on the subjects' individual sleep patterns. Those in the major depressive disorder group had a score of at least 18 on the HAM-D17. Cortisol was measured by radioimmunoassay. They found that the depressed subjects had a significantly higher cortisol level than the control subjects at the first time point of the day (8:00 am). The higher cortisol levels also correlated with impaired cognition.

A subsequent study by Hinkelmann et al (2011) showed that 3 weeks of treatment with escitalopram, an antidepressant, lowered cortisol levels in depressed patients

significantly so that the cortisol levels were equivalent to those of healthy controls. Depression severity as measured by the HAM-D also significantly improved. Further, the decrease in cortisol was significantly correlated with the decrease in HAM-D score.

Vreeburg et al (2009) compared salivary cortisol among currently depressed (n=701), previously depressed (n=579), and healthy subjects (n=308). They assessed the CAR by measuring salivary cortisol at the time of awakening and at 30, 40, and 60 minutes following awakening. Evening cortisol was measured at 2000 h and 2100 h. Cortisol was measured using competitive electrochemiluminescence immunoassay. Depression severity was measured using the Inventory of Depressive Symptoms. The currently-depressed subjects and the subjects with a history of major depressive disorder had significantly higher cortisol levels than healthy controls in the hour after awakening. In addition, the currently-depressed subjects had a significantly higher cortisol level at the 2000 h evening timepoint. In a subsequent study, Manthey et al. (2011) used the same experimental design to examine cortisol in subjects taking antidepressants. Subjects taking selective serotonin reuptake inhibitors (SSRIs; n=309) did not have a significantly different CAR from the healthy controls (n=1068); however, subjects taking tricyclic antidepressants (TCAs; n=49) showed a flattened CAR measured by area under the curve (AUC). Evening cortisol levels were significantly higher in SSRI and TCA users than in healthy controls.

Burgese and Bassitt (2015) examined the effect of electroconvulsive therapy (ECT) on plasma cortisol levels in depressed patients. Depression severity was assessed using the Beck Depression Inventory (BDI), and morning cortisol was measured using the Immulite 2000. Depressed subjects (n=11) had their morning cortisol and BDI

measured before the first ECT session, after the 7th ECT session, and after the last ECT session. Cortisol and BDI were measured at one timepoint in control subjects. Cortisol level and BDI score were significantly higher in the depressed group compared to the control group. While BDI scores significantly decreased after ECT therapy, cortisol levels did not significantly decrease.

Jia et al (2019) recruited 55 adult male inpatients with depression and 34 men without depression. Depression severity was measured using the 17-item Hamilton Depression Rating Scale (HAMD), and anxiety was measured using the Beck Anxiety Inventory (BAI). Serum cortisol was collected between 0600 h and 0900 h and measured using an iodine cortisol radioimmunoassay. There was a significant positive correlation between cortisol levels and HAMD scores. There was a significant difference in cortisol level between the control group and each of the three levels of depression severity (mild, moderate, and severe). There was also a significant difference in cortisol level between the mild and severe depression subgroups.

Cortisol elevation in association with depression has not been observed in all studies. Krogh et al. (2012) examined salivary cortisol levels between mildly- and moderately-depressed subjects and healthy controls at three timepoints throughout the day. They used a competitive radioimmunoassay to measure cortisol. Depression was assessed by the research staff using the Medical Depression Inventory (MDI), while severity of depression was measured using the HAM-D17. They found no significant differences in cortisol levels between the two groups at any of the timepoints.

Schatzburg et al. (2014) examined genetics in association with serum cortisol and MDD with and without psychosis. They recruited 40 patients with psychotic depression,

26 patients with nonpsychotic depression, and 29 healthy controls. Depression severity was assessed using the HAM-D, and psychosis status was determined by the Brief Psychotic Rating Scale. Depressed patients remained on their medication regimen, which included treatment for their depression, but subjects who were on medication that interfered with serum cortisol levels (such as prednisone, oral contraceptives, and hormone replacement therapy) were excluded from the study. Blood was collected hourly from 1800 to 0900 the next day, and cortisol was measured from these samples. Those with depression alone did not have significantly different mean cortisol levels than healthy controls, but those with depression and psychosis had significantly higher mean cortisol levels.

Depression frequently occurs in association with anxiety. In fact, anxiety is one of the symptoms of depression (National Institute of Mental Health, 2019). Veen et al. (2009) examined salivary cortisol in 42 healthy controls and 72 patients with depression, anxiety, or both. Cortisol diurnal curves were constructed for each subject using 8 samples through the day: at awakening, 30, 45, and 60 minutes after awakening, 1100 h, 1500 h, 1900 h, and 2300 h. Sampling was repeated 8 times over a second day for each subject. Cortisol analysis was performed using competitive electrochemiluminescence assay. Depressed/anxious patients showed a higher AUC.

A large Danish longitudinal study examined the correlation between subjective stress and salivary cortisol. Mikkelsen et al. (2017) followed a cohort of 3217 Danish public service employees over 2 years. Subjects collected saliva 30 minutes after waking and at 2000 h, and salivary cortisol was determined using radio immunoassay. Subjects also performed a questionnaire that included a measure of perceived stress using a Danish

version of Cohen's perceived stress scale (PSS-4). Surprisingly, no significant correlations between cortisol and perceived stress were seen.

**Depression and Cortisol in Higher Education**

There have been numerous studies on depression, anxiety, and cortisol specifically in college student populations. McGregor, Murphy, Albano, and Ceballos (2016) examined salivary cortisol levels and stress and depression in first year graduate students (n=24) and control non-students (n=34). Depression status was assessed using the CES-D. Cortisol levels were assessed before classes started for the semester (T1) and again 5 days before qualifying exams (T2). Cortisol was measured at 5 timepoints during each day: waking, 30 minutes after waking, 11 am, 3 pm, and bedtime. Cortisol level was determined using chemiluminescence immunoassay. Area under the curve and CAR were calculated. The CAR was significantly flattened in the second time point in the students; at the timepoint 30 minutes after wakening, salivary cortisol was significantly lower than that of controls and the students at T1. However, depression severity measures in students at T1 and T2 were practically identical, as were stress severity measures.

Drake, Sladek, and Doane (2016) also examined salivary cortisol in students in their first semester of college. The researchers correlated cortisol levels to loneliness, which is associated with depression. Seventy participants collected a saliva sample 5 times a day for 3 consecutive days in the spring of their senior year of high school (T1) and again during the fall of their first semester of college (T2). They also completed the UCLA Loneliness Scale Version 3 at T1 and T2. Those who scored the highest in loneliness at T2 showed a higher slope of the diurnal cortisol curve.

Another study examined the correlation of salivary cortisol levels with depression and loneliness. Lai, Leung, Lee, and Lam (2018) examined the CAR, AUC, and diurnal slope (the slope of the curve between evening cortisol level and waking cortisol) in 51 Chinese undergraduates. They measured depression using a Chinese version of the CES-D and measured loneliness using a Chinese version of the eight-item Loneliness Scale. Cortisol was measured in salivary samples using an enzyme-linked immunosorbent assay. Depression severity was not correlated with any of the indices of diurnal cortisol. However, higher loneliness scores were significantly correlated with an attenuated CAR, a larger AUC, and a steeper diurnal slope.

In a 2011 study, Kenwright et al. compared salivary cortisol at two timepoints in 23 physical therapy students. The first timepoint took place at the beginning of the first semester, which was deemed a low-stress period, and the second timepoint took place during the second semester the day preceding an exam. Each timepoint consisted of a morning collection between 0730 h and 0800 h and an evening collection between 1930 h and 2030 h. Cortisol levels were determined using enzyme immunoassay. The morning cortisol level during the second semester (stressful timepoint) was significantly lower than that of the morning cortisol during the first semester. The difference in the morning and evening cortisol (calculated by subtracting evening cortisol level from morning cortisol level) was significantly greater during the second semester timepoint.

Baker et al. (1985) examined serum cortisol levels and subjective anxiety levels in first- and second-year medical students at two timepoints. Blood was drawn between 1100 h and 1300 h. The first timepoint was in October, and the second timepoint was approximately 4 months later. Timepoint one of the first-year students (n=33) yielded

significantly higher mean serum cortisol level and mean anxiety score than second-year students at timepoint one (n=28), second-year students at timepoint two (n=22), and timepoint two of the first-year students (n=31).

A 2015 study examined the association between exam stress and cortisol in 208 Nigerian college undergraduates. Maduka, Naboh, and Ufelle compared serum cortisol obtained 1-3 hours immediately before an exam and 3-4 weeks before an exam and found that cortisol was significantly higher during the period immediately preceding an exam.

These studies indicate that there may be a positive correlation between cortisol concentration and depression in certain groups of people. However, this relationship has not been explored in the college freshman population. The current study aimed to determine whether serum cortisol concentration is an effective biomarker for depression in college freshmen.

**Patient Health Questionnaire**

The Patient Health Questionnaire (PHQ) 9-item (referred to as PHQ-9) is a tool used to diagnose and measure severity of depression. It consists of 9 items that correspond to the 9 criteria in the Diagnostic and Statistical Manual of Mental Disorders, 4$^{th}$ Edition (DSM-IV) for diagnosing depression:

1. Little interest or pleasure in doing things
2. Feeling down, depressed, or hopeless
3. Trouble falling or staying asleep, or sleeping too much
4. Feeling tired or having little energy
5. Poor appetite or overeating

6. Feeling bad about yourself – or that you are a failure or have let yourself or your family down

7. Trouble concentrating on things, such as reading the newspaper or watching television

8. Moving or speaking so slowly that other people could have noticed. Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual

9. Thoughts that you would be better off dead or of hurting yourself in some way

For each item, the responder indicates how often he/she has been bothered by the item over the previous 2 weeks (not at all, several days, more than half the days, or nearly every day). Each response is assigned a point score from 0 to 3, and the points are added up for an overall score of 0 to 27. The higher the score, the more severe the depression (Spitzer, Kroenke, and Williams, 1999). The score ranges and corresponding depression categories are shown in Table 2.1.

| PHQ-9 score | Depression category |
|---|---|
| 0-4 | None/minimal |
| 5-9 | Mild |
| 10-14 | Moderate |
| 15-19 | Moderately-severe |
| 20-27 | Severe |

*Table 2.1.* PHQ-9 scores and corresponding depression categories.

The PHQ-9 was developed for use as a screening tool for depression in primary care settings. Its brevity and ease of scoring and interpretation are main advantages. It has been validated in many populations. The creators of the questionnaire completed a large

study using patients in 7 primary care clinics and 8 obstetrics-gynecology clinics and found that a score of at least 10 on the PHQ-9 had a sensitivity of 88% and a specificity of 88% for major depression (Kroenke, Spitzer, and Williams, 2001).

Keum, Miller, and Inkelas (2018) confirmed the validity of the PHQ-9 in college students in the United States. The study population consisted of 857 ethnically-diverse U.S. college students from 51 colleges and universities. The researchers found the PHQ-9 to be reliable and valid in this population.

The current study examined potential correlations between PHQ-9 score and serum cortisol concentration in college freshmen. Any correlations may indicate that serum cortisol has utilization as a biomarker for depression in college freshmen.

# Chapter III: Methodology

## Participants

Fifty Idaho State University (ISU) freshmen were recruited for the study at the beginning of the fall semester. The majority of participants were recruited in person at the ISU Freshman Orientation Resources Fair. The rest were recruited through the use of flyers containing information about the study and directions for signing up for the study. The flyers were approved by the ISU Marketing and Communications Office and posted in many ISU campus buildings, such as Reed Gym, Oboler Library, the Student Union Building, and many other buildings where classes are held. Flyers were also posted in off-campus restaurants near the ISU campus, such as 5th Street Bagelry, Mocha Madness, and Goody's. In order to qualify for participation in the study, the student was required to fulfill all of the following criteria:

- Age of 18 or older
- Full-time student status (course load of at least 12 credits) during the semester of the study
- Freshman status (maximum of 25 total credits earned)
- Idaho resident

## Study Approval

All procedures were approved by the Idaho State University Institutional Review Board.

## Informed Consent

Before the initiation of any experimental procedures, participants were informed of the purpose of the study and the procedure and timeline. They were told that

participation was voluntary and that they could withdraw from the study at any time. Risks of the study, such as risks associated with venipuncture and potential data confidentially breaches, were explained to the participants. Compensation for completing the study was outlined for the participants. If the participants wanted to proceed with the study, they signed the Informed Consent form.

**Procedure**

Session One took place during the first 3 weeks of the fall semester. Each student was scheduled for one morning session and one evening session. The morning session took place between 0600 h and 1030 h, and the evening session took place between 1500 h and 2000 h. At the morning session, the student completed the PHQ-9 and a short questionnaire for demographic information and history of depression and stress. Then blood was obtained from the student through venipuncture in serum separator tubes for cortisol analysis. At the evening session, blood was again obtained in serum separator tubes for cortisol analysis. Session Two took place during 3 weeks near the end of the fall semester. The methods for the morning and evening sessions were the same as during Session One.

**Materials**

**Venipuncture.** For the venipuncture, the phlebotomist's hands were sanitized using hand sanitizer, and nitrile gloves were donned. A tourniquet (catalog no. 21-520-130) was tied around the upper arm of the subject. A suitable vein, usually the median cubital vein or cephalic vein, was located using palpation with the finger. The skin over the vein was then sanitized using a sterile isopropanol prep pad (Dynarex, catalog no. 1113). After the isopropanol on the skin was dry, a sterile 21 gauge, 1-1/4-inch long

needle (BD Vacutainer catalog no. 368607) with a tube holder (BD Vacutainer, catalog no. 364815) attached was inserted into the vein. A 5.0 ml serum separator tube (BD Vacutainer SST, catalog no. 367986) was then placed in the tube holder and left in place until blood was no longer flowing into the tube. The tourniquet was then removed from the arm, and then the tube was removed from the tube holder and gently inverted 5 times. The needle/tube holder apparatus was removed from the vein, and a square of gauze (Dynarex, catalog no. 3223) was placed on the vein using pressure to promote the stopping of bleeding. The needle was disposed of immediately in a sharps container (Fisherbrand, catalog no. 22037959), and the subject's venipuncture site was wrapped with a self-adhesive bandage (Dynarex Sensi-Wrap, catalog no. 3181).

**Centrifuge.** The blood was allowed to sit for at least 10 minutes to allow for the blood to clot. The serum separator tubes were then spun using a ThermoScientific Medifuge.

**Facility**

All experimental procedures, including the completion of questionnaires and venipuncture, were performed in the Bengal Lab on the ISU campus. The Bengal Lab is a Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratory.

**Participant Incentive**

Participants were compensated $10 for completion of the questionnaires, $10 for completion of the morning venipuncture, and $10 for completion of the evening venipuncture, for Sessions One and Two. Total compensation for a participant completing the entire study was $60.

**Cortisol analysis**

Blood was collected in serum separator tubes and centrifuged for 10 minutes at 5000 RPM within 2 hours of collection. Samples were stored refrigerated until analysis, which occurred no longer than 3 days after sample collection. Total serum cortisol was measured using competitive binding immunoenzymatic assay.

**Patient Health Questionnaire 9**

The PHQ-9 is a 9-item questionnaire based on the 9 DSM-IV criteria for depression and has excellent reliability and validity (Kroenke, Spitzer, and Williams, 2001). Its validity has also been confirmed in university students in the United States (Keum, Miller, and Inkelas, 2018).

**Statistical analysis**

Spearman's rho was used to evaluate intercorrelations between nonparametric measures within each session. The Mann-Whitney U test was used to evaluate the correlation between depression history and PHQ scores and cortisol concentrations within each session. The Wilcoxon rank sum test was used to evaluate changes from Session 1 to Session 2. These tests do not assume that the data are normally distributed.

## Chapter IV: Results

Demographic information of participants is shown in Table 4.1. Of the participants that participated in both Sessions One and Two, 32 (71%) were female, and 13 (29%) were male. The participants ranged in age from 18 to 72 years with the average age being 20.1 years. Self-reported ethnicities of participants are listed in Table 4.2. The majority of participants (n=31, 68%) identified as white. The second most common ethnicity reported was "Hispanic or Latino/a," and 6 participants (13%) identified as this.

| | Mean age (years) | Depression history |
|---|---|---|
| Overall (n=45) | 20.1 | 12 (27%) |
| Female (n=32) | 20.4 | 9 (28%) |
| Male (n=13) | 19.5 | 3 (23%) |

*Table 4.1.* Sex, mean age, and depression history of participants.

| Ethnicity | N (%) |
|---|---|
| White | 31 (68%) |
| Hispanic or Latino/a | 6 (13%) |
| White, Hispanic or Latino/a | 3 (0.07%) |
| Asian or Pacific Islander | 2 (0.04%) |
| White, Hispanic or Latino/a, American Indian, Alaskan Native, or Native Hawaiian | 1 (0.02%) |
| White, Asian or Pacific Islander | 1 (0.02%) |
| Biracial or Multiracial | 1 (0.02%) |

*Table 4.2.* Ethnicities of the 45 participants that completed the entire study.

Out of the 45 participants completing the study, approximately half scored in the PHQ-9 depression category of "no depression/minimal" in Sessions One and Two (Figure 4.1). The number of participants scoring in each depression category is shown in Table 4.3.

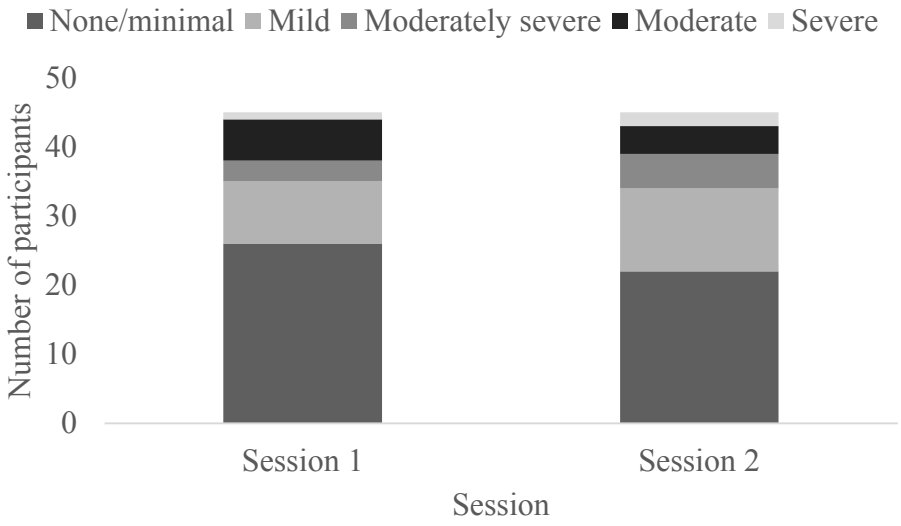


*Figure 4.1.* Number of participants scoring in each depression category of the PHQ-9 by session

| | PHQ9 Depression Category | | | | |
|---|---|---|---|---|---|
| | None/minimal | Mild | Moderate | Moderately severe | Severe |
| Session 1 | 26 (57%) | 9 (20%) | 3 (6.7%) | 6 (13%) | 1 (2.2%) |
| Session 2 | 22 (49%) | 12 (27%) | 5 (11%) | 4 (8.9%) | 2 (4.4%) |

*Table 4.3.* Number of participants scoring in each depression category of the PHQ-9 by session

There were no significant differences in morning or evening cortisol concentration from Session One to Session Two (data not shown). There was no significant correlation between PHQ9 score and morning (Figures 4.2 and 4.3) or evening serum cortisol concentration (Figures 4.4 and 4.5) during either Session One or Session

Two. Similarly, there was no significant correlation between PHQ9 score and evening/morning cortisol ratio during either session 1 (Figure 4.6) or session 2 (Figure 4.7).

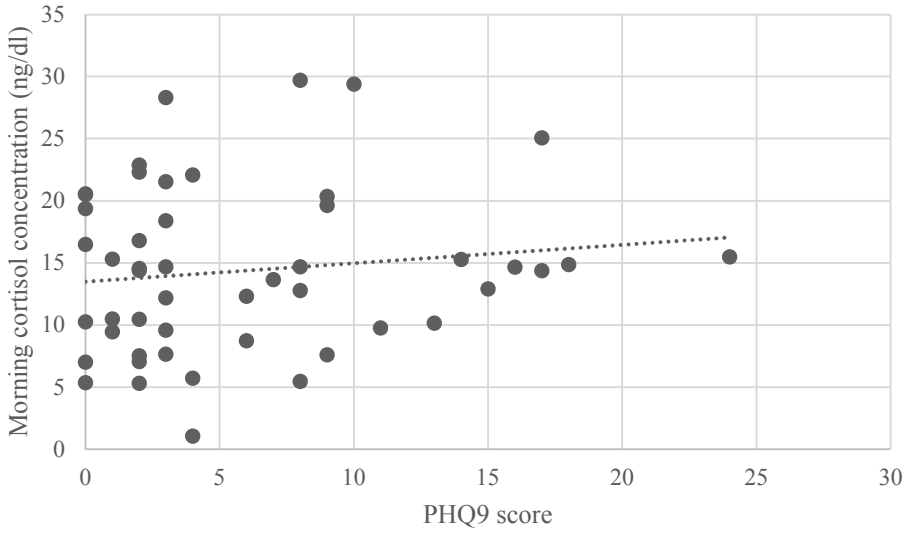


*Figure 4.2.* Session 1 correlation between morning cortisol concentration and PHQ-9 score. Dotted line is a best-fit line.

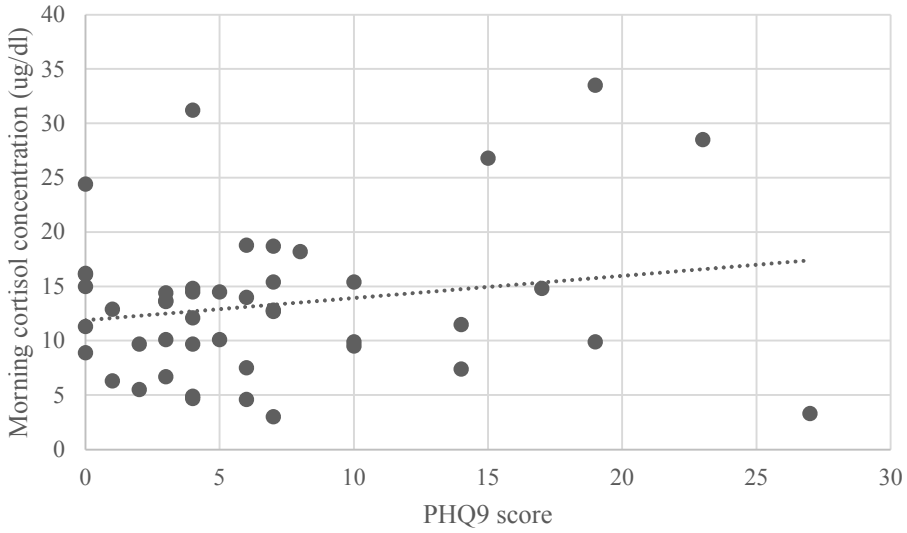


*Figure 4.3.* Session 2 correlation between morning cortisol concentration and PHQ-9 score. Dotted line is a best-fit line.

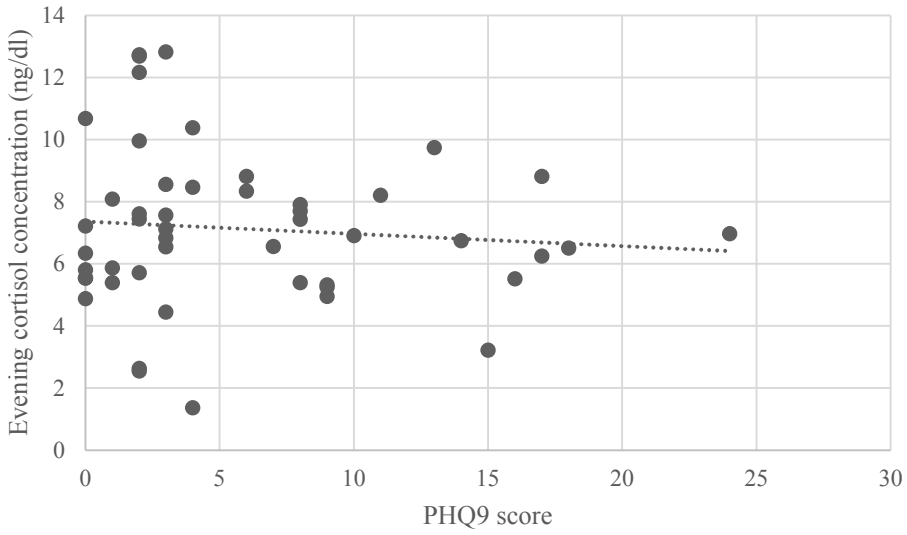


*Figure 4.4.* Session 1 correlation between evening cortisol concentration and PHQ-9 score. Dotted line is a best-fit line.

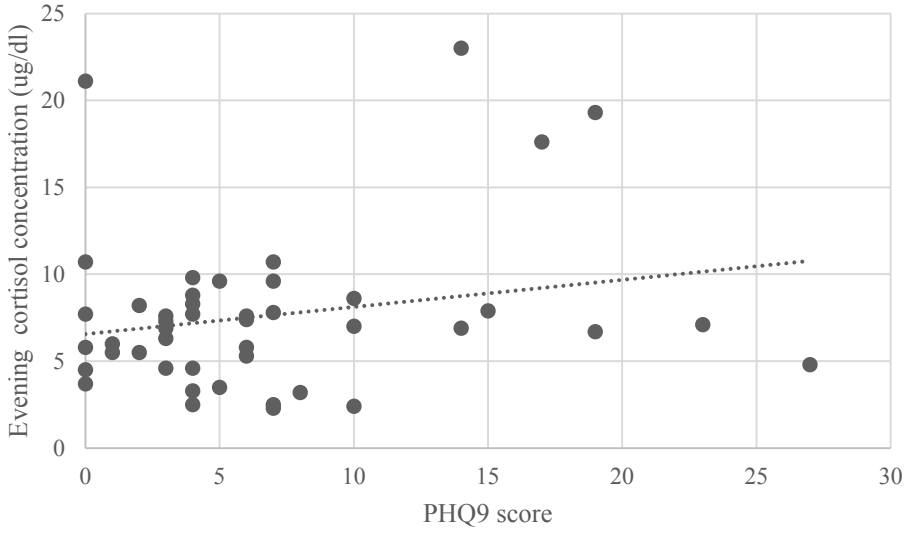


*Figure 4.5.* Session 2 correlation between evening cortisol concentration and PHQ-9 score. Dotted line is a best-fit line.

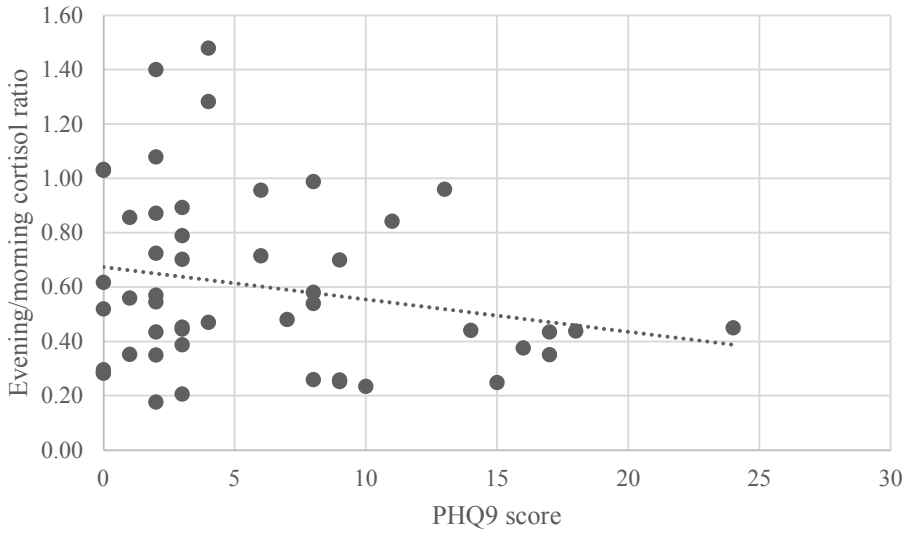


*Figure 4.6.* Session 1 correlation between evening/morning cortisol ratio and PHQ-9 score. Dotted line is a best-fit line.

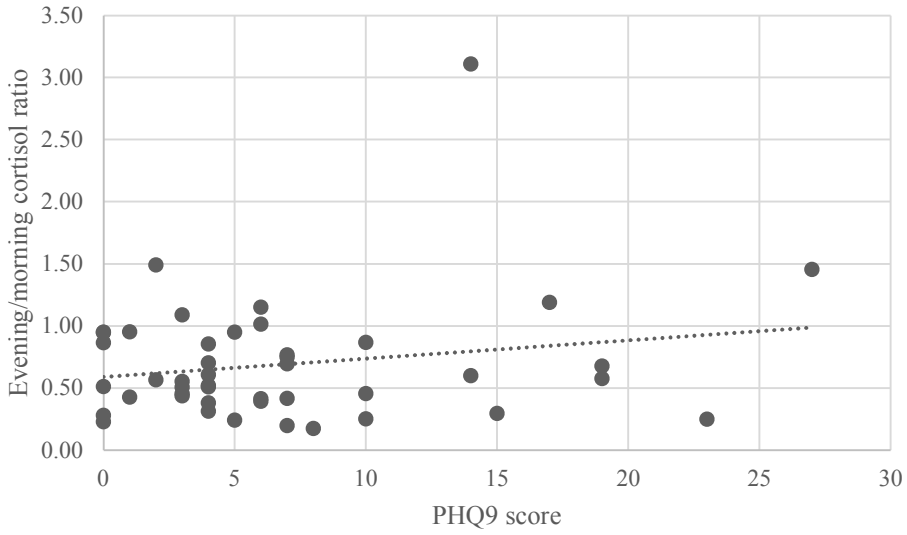


*Figure 4.7.* Session 2 correlation between evening/morning cortisol ratio and PHQ-9 score. Dotted line is a best-fit line.

As shown in Figures 4.8 and 4.9, there were no significant differences in morning or evening serum cortisol concentration between those with no history of depression and those with a current or past depression diagnosis. This was true for both Session One (Figure 4.8) and Session Two (Figure 4.9).

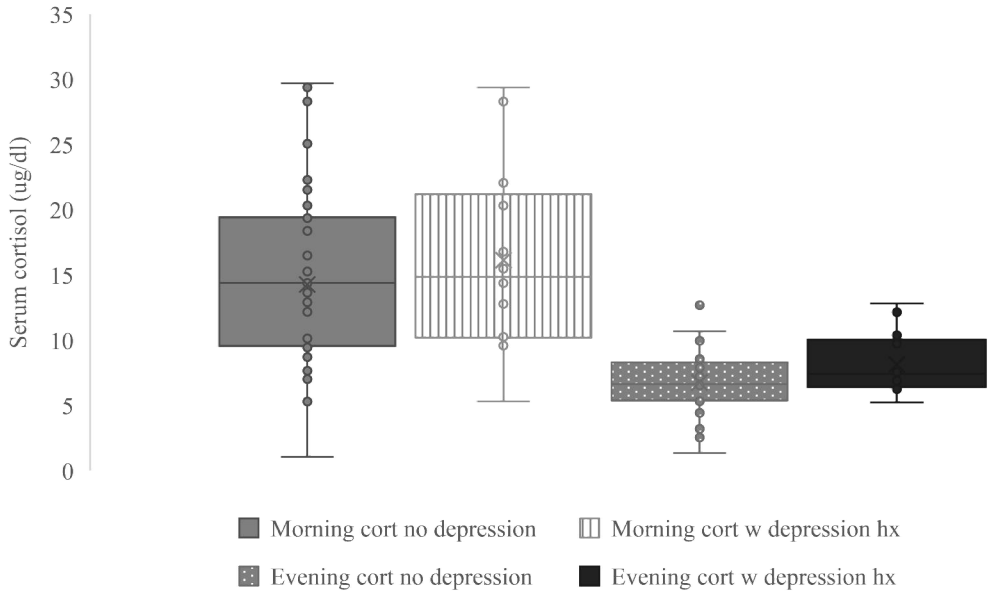


*Figure 4.8.* Session 1 serum cortisol concentration by history of depression diagnosis. Horizontal lines within the boxes represent the medians. Upper and lower boundaries of the boxes represent the interquartile range (75$^{th}$ and 25$^{th}$ percentiles, respectively). Whiskers represent "minimum" (1.5*interquartile range below the 25$^{th}$ percentile) and "maximum" (1.5*interquartile range above the 75$^{th}$ percentile). Dots represent individual data points.

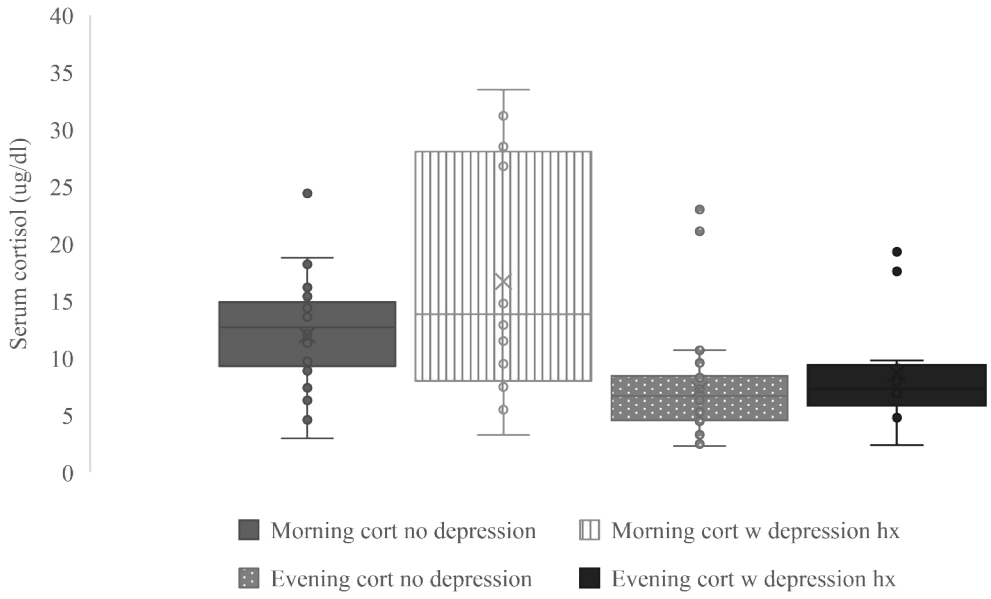


*Figure 4.9.* Session 2 serum cortisol concentration by history of depression diagnosis. Horizontal lines within the boxes represent the medians. Upper and lower boundaries of the boxes represent the interquartile range ($75^{th}$ and $25^{th}$ percentiles, respectively). Whiskers represent "minimum" (1.5*interquartile range below the $25^{th}$ percentile) and "maximum" (1.5*interquartile range above the $75^{th}$ percentile). Dots represent individual data points.

The mean PHQ9 score did not change significantly from Session One to Session Two (Figure 4.10). In Session Two, those participants with either a history of or a current diagnosis of depression had significantly higher (alpha=0.05) sum of ranks of PHQ9 scores than those of the participants with no history of depression (Figure 4.11). This significant difference was not observed during Session One.

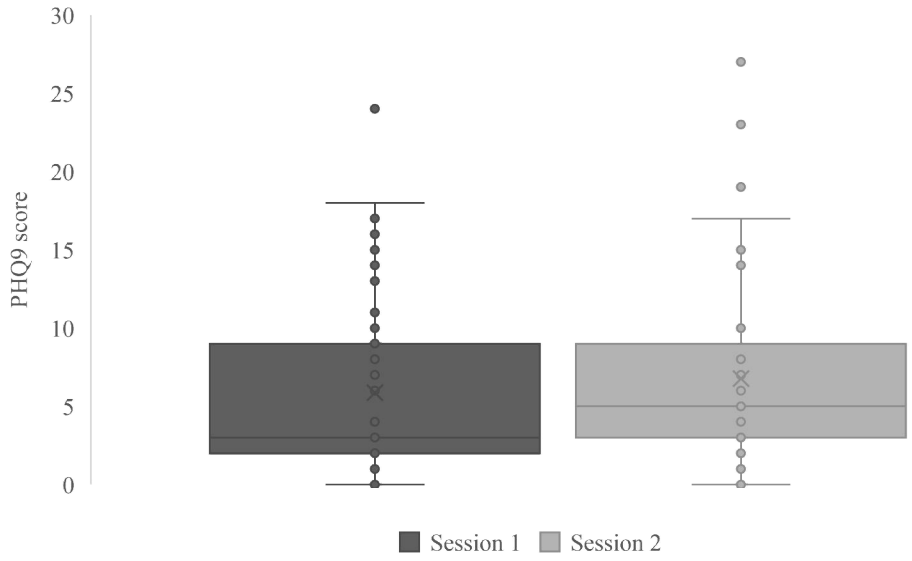


*Figure 4.10.* PHQ-9 scores by session. Horizontal lines within the boxes represent the medians. Upper and lower boundaries of the boxes represent the interquartile range (75th and 25th percentiles, respectively). Whiskers represent "minimum" (1.5*interquartile range below the 25th percentile) and "maximum" (1.5*interquartile range above the 75th percentile). Dots represent individual data points.

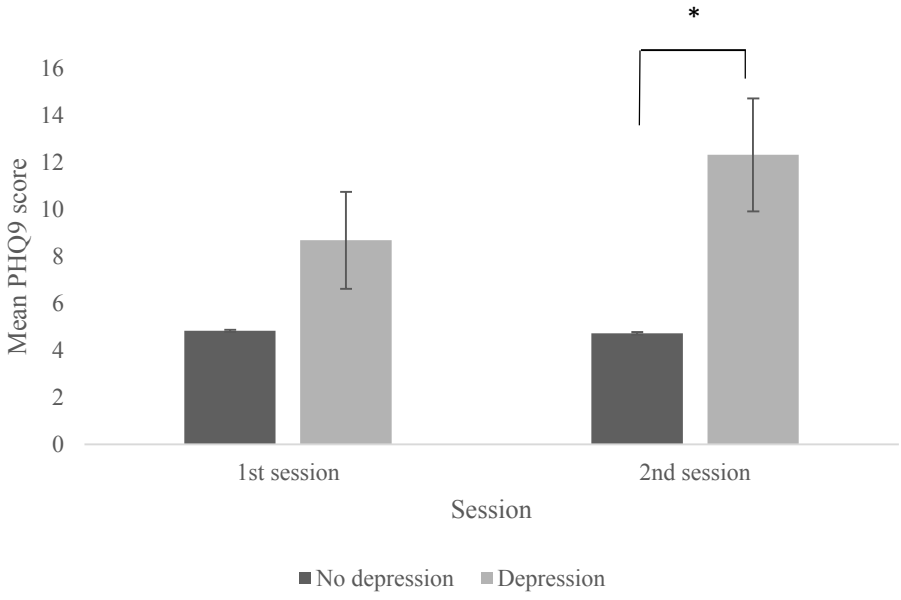


*Figure 4.11.* Mean PHQ-9 scores for each session by history of depression. Bars are +/- standard error of the mean. Asterisk signifies significance at alpha = 0.05.

# Chapter V: Conclusion

## Discussion

The results of our study show no significant correlation between serum cortisol concentration and depression severity as measured by PHQ-9 in our subject group of freshmen at ISU. There were no significant correlations between PHQ-9 scores and morning cortisol concentration, evening cortisol concentration, or evening/morning cortisol concentration ratio.

Our finding that cortisol did not correlate with PHQ9 score agrees with a study by Krogh et al. (2012), who found no increase in salivary cortisol levels in mildly- and moderately-depressed subjects. However, it conflicts with a more recent study by Jia et al. (2019), who found significantly elevated serum cortisol levels in patients with mild, moderate, and severe depression. The discrepant findings may be explained by differences in the two patient populations. While Krogh et al. (2012) examined outpatients with mild and moderate depression, Jia et al. (2019) examined inpatients, which possibly comprises a group of more severely-depressed patients despite their HAMD-17 depression scores indicating less-severe depression.

Approximately half of our participants scored in the “no depression/minimal” category of the PHQ-9 in both sessions. During Session One, only one subject scored in the “severe depression” category, and during Session Two, only two subjects scored in the “severe depression” category. Among those scoring positive for depression, most scored in the “mild depression” category. It is possible that serum cortisol concentration is not sensitive enough to detect depression in those with mild or moderate depression.

Twenty-seven percent of our subjects reported that they had been diagnosed with depression. This is slightly higher than the 24.9% reporting a history of depression in a survey of college students by the American College Health Association in 2019. Our finding of a rate of 27% reported history of depression is very similar to that of Lipson, Lattie, and Eisenberg (2019). In their large-scale study of 196 U.S. college campuses, they found a 29.9% prevalence of depression as measured by the PHQ-2, a 2-item version of the PHQ-9.

The PHQ-9 scores of our subjects were also comparable to reported rates of depression among college students in the U.S. A score of greater than 10 has been shown to have a high correlation with the presence of depression, while scores below 10 are rarely observed in those with active, symptomatic depression (Kroenke, Spitzer, and Williams, 2001).The percentage of subjects in the present study scoring above 10 on the PHQ-9 was 22% during Session One and 24% during Session Two.

During Session Two (near the end of the fall semester), those subjects who reported a history of a depression diagnosis had a significantly higher mean PHQ-9 score than those subjects who reported no history of depression. Those with no history of depression had a mean PHQ-9 score of 4.7, while those with a history of depression had a mean score of 12.3. This supports the reliability and validity of the PHQ-9 in a college student population that has previously been demonstrated by Keum, Miller, and Inkelas (2018).

## Limitations

A major limitation in our study was the timing of the morning blood draw. The CAR is highly linked to the time of waking, so the cortisol concentration depends greatly on the time that has elapsed since waking. Our study included only a single morning blood draw. Further, the timing of the blood draw was not dependent on the time that the participant awoke that day. The only timing requirement for the morning blood draw was that it be between 0600 h and 1100 h. This means that our cortisol measurements were taken at different points in the subjects' CARs, which makes it extremely difficult to make comparisons between subjects. Future studies would benefit from standardizing the collection time based on the individual subject's waking time. The ease of sampling is an advantage to using saliva collection for cortisol measurements; saliva collection can then be done by the subjects in their own homes immediately upon waking.

Additionally, measuring cortisol at more than one time point in the morning would allow for the generation of the CAR curve and for the detection of differences in the CAR curve. This sampling technique was used by Doolin et al. (2017), who found that those with major depression showed a more shallow CAR when compared to healthy subjects. A disruption in the CAR was also observed in patients taking TCAs for depression in a study by Manthey et al. (2011). In this case, the CAR was also flattened.

## Assumptions

We made some significant assumptions about our subjects. First, we assumed that our subjects were on normal sleep schedules: that is, that our subjects awoke in the morning hours between 0600 h and 0900 h and went to sleep at night between

approximately 2100 h and midnight. We assumed that no subjects worked shifts outside of normal business hours (e.g., swing shifts or graveyard shifts). Working these shifts would greatly affect the subject's sleep cycle and therefore cortisol rhythm.

We also made an assumption that our subjects were not taking medications that could affect serum cortisol level or could interfere with the testing of serum cortisol level. However, many medications have been shown to affect cortisol level, and some of them are quite common. These include contraceptives (Drake et al., 2016), steroid treatments for inflammatory conditions (Kenwright et al., 2011), and antidepressants (Hinkelmann et al., 2012).

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