STRESS, BULLYING, CORTISOL, AND DEPRESSIVE SYMPTOMS
IN 9TH GRADE ADOLESCENTS

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STRESS, BULLYING, CORTISOL, AND DEPRESSIVE SYMPTOMS IN 9TH GRADE ADOLESCENTS

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ABSTRACT

Stress and bullying are known contributors to depressive symptoms in adolescents. Prevalence of depressive symptoms in adolescents is estimated to range from 13% to 34% in recent studies. Ninth graders are in a transitional period developmentally, biologically, physically, and psychosocially. Few studies have examined the relationship between stress, bullying, and depressive symptoms from a biobehavioral perspective in 9th graders. The purpose of this study was to examine stress, bullying, and depressive symptoms including a biomarker of stress, salivary cortisol diurnal rhythm, to determine if there is mediation between the individual variables of stressful life events, perceived stress, bullying, and depressive symptoms. The theoretical framework was McEwen’s Theory of Allostatic Load.

A convenience sample of 9th graders (n = 143), was recruited from two high schools in a Southern state. Saliva specimens for cortisol were collected in the morning and afternoon. After collection of the morning saliva sample, adolescents completed online surveys, including the Center for Epidemiologic Studies Depression Survey-10, Perceived Stress Scale-10, Personal Experiences Checklist, and Self Rating Scale for Pubertal Development. An afternoon saliva sample was collected, and adolescents’ completed the Coddington Life Event Scale-Adolescents by paper and pencil.
Adolescents scoring > 9 on the depression survey (clinically significant depressive symptoms) were referred to the school nurse.

Approximately 40% of adolescents scored 10-30 or clinically meaningful depressive symptoms. Stressful life events ranged from 0-79 events in one year. Life change unit scores ranged from 0-1691 with 80% of participants scoring above the normative score of 170. Perceived stress scores revealed that 84% of adolescents scored 10-30 out of a 40-point scale. Bullying scores revealed an average score of 15.14 out of 128 total score. Significant correlations with depressive symptoms were noted with stressful life events, life change units, perceived stress, verbal/relational bullying, and gender identity. Two variables (PSS-10, PECK) and one covariate (gender identity) were significant predictors of depressive symptoms. These findings should help to guide school nurses, teachers, counselors, or other professional working with this population. Future research is warranted with 9th graders experiencing a transition to high school.

Keywords: stressful life events, life change units, perceived stress, bullying, cortisol diurnal rhythm, depressive symptoms, and 9th grade adolescents
DEDICATION

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CHAPTER ONE

INTRODUCTION

The current dissertation research study examined stress, bullying, and depressive symptoms in 9th grade adolescents. Stress variables included stressful life events, perceived stress (PSS-10), and cortisol (a stress hormone). This chapter provides an overview of the issues surrounding these variables with the goal of identifying the physiological relationships that occur among stress, bullying, and depressive symptoms in a vulnerable population of adolescents. Covariates examined were gender, race, socioeconomic status (SES), gender identity, and pubertal status.

Depressive symptoms generally represent a significant difference in the way an individual functions on a daily basis. At least some of the following characteristics of depressive symptoms are identified as: changes in sleep, changes in appetite, poor concentration, loss of energy, lack of interest, low self-esteem, hopelessness or guilt, and/or slow movement with physical depletion (The National Alliance on Mental Illness [NAMI] 2015).

According to the National Comorbidity Survey – Adolescent Supplement, 11% of adolescents experience depressive symptoms by age 18, with females experiencing symptoms more frequently than males (Kessler et al., 2005; Merikangas, Avenevoli, Costello, Koretz, & Kessler, 2010). Furthermore, NAMI (2015) reports that half of all mental health disorders begin by age 14 with one in ten Americans describing at least one episode of depression (Centers for Disease Control and Prevention [CDC], 2013).
Depressive symptoms often present in childhood or adolescence (Abela & Hankin, 2008; Kessler, Avenevoli & Merikangas, 2001), and long term studies that follow adolescents into adulthood indicate that there is high risk for recurrent episodes of depression to occur when the symptoms of depression begin at an early age (Lewinsohn, Solomon, Seeley, & Zeiss, 2000). Adolescence is a key developmental time involving biological, psychological, and social changes (Dahl & Gunnar, 2009) that may coincide with life events such as divorce of parents, illness of a family member, moving, and many other issues that are perceived as stressful to an adolescent (Coddington, 1972). In addition, adolescents may experience stress due to challenging academics, extracurricular activities, and relationships (peers, romantic interests, family), risk-taking behaviors, and emotional sensitivities, all of which are a typical part of this developmental period (McHale et al., 2012; Siegel, 2012).

When adolescents begin high school in the 9th grade, they generally experience more independence as they spend less time with parents and more time outside the home with peers (Allen et al., 2006; Friedlander, Connolly, Pepler, & Craig, 2007). In addition to peer relationships, romantic relationships tend to be more intense both emotionally and sexually during the high school years (Connolly, Craig, Goldberg, & Pepler, 2004). Rejection from peers or instability in romantic relationships can lead to depressive symptoms depending on the intensity of the relationship, other friendships, and sexual activity with a romantic partner (Chango, McElhaney, Allen, Schad, & Marston, 2012; Davila, 2008; Hall-Lande, Eisenberg, Christenson, & Neumark-Sztainer, 2007; Marston, Hare, & Allen, 2010; Masten, Telzer, Fuligni, Lieberman, & Eisenberger, 2012; Taradash, Connolly, Pepler, Craig, & Costa, 2001).
Adolescents may have stressful experiences both inside and outside of school including peer pressure related to risky behaviors such as sexual relationships, smoking, alcohol, or involvement with drugs (Patton et al., 2004). If an adolescent refuses to participate in risky behaviors, this may then lead to lack of acceptance in some social circles, thus, a potential lack of social support (Flaspohler, Elfstrom, Vanderzee, Sink, & Birchmeier, 2009). Researchers have documented that bullying occurs during the high school years, more so in the 9th grade than in 10th, 11th, or 12th grades (Langdon & Preble, 2008; Peskin, Tortolero, & Markham, 2006). Bullying is considered a stressful experience and is also related to an increase in general mental health issues with internalizing symptoms such as anxiety, loneliness, low self-worth, and depressive symptoms (Feliz & McMahon, 2006).

When a stressful life event occurs, the adolescent’s perception of stress can lead to a complex interconnection between the body and the mind. This process can be measured quantitatively by examining the cortisol diurnal rhythm, which is the end point of the hypothalamic pituitary adrenocortical (HPA) axis, a critical area in the brain related to stress (Hazler, Carney, & Granger, 2006). This study sought to determine if this unique hormonal pattern (cortisol diurnal rhythm) mediates the relationship between stressful life events, perceived stress, bullying, and depressive symptoms.

In summary, the biological, social, and emotional issues faced by an adolescent may be linked to depressive symptoms. The relational situations that occur, both acceptance and rejection, may also lead to depressive symptoms (Humensky et al., 2010; Marston et al., 2010). If an adolescent continues to experience depressive symptoms upon becoming an adult, these symptoms may lead to periods of inability to work or to be
productive that may in time lead to increased long-term disability, incurring a cost to society (Chen, Haas, Gilmore, & Kopak, 2011; Garber, Keiley, & Martin, 2002). In a recent report by the Agency for Healthcare Research and Quality (AHRQ) (2014), the highest expenditure for care and treatment in 2011 for children and adolescents was found to be related to mental health disorders.

Researchers are beginning to use innovative methods such as analysis of a physiological marker (salivary cortisol) to examine mental health issues in adolescents (Burghy et al., 2012; Gunnar, Wewerka, Frenn, Long & Griggs, 2009). Georgiades, Lewinsohn, Monroe and Seeley (2006) posit that identifying “subthreshold depression” or depressive symptoms is important as these symptoms may often lead to major depressive disorder (p. 936). There is a paucity of research with 9th grade adolescents with regard to stress, the adolescent’s perception of stress, and the physiological related factors to depressive symptoms. By identifying depressive symptoms earlier, possible systems of dysregulation may be recognized, providing a prevention strategy for major depression in the future (Georgiades et al., 2006). Furthermore, referrals may occur sooner than in years past, which could possibly change the trajectory of quality of life for an adolescent (Mihalopoulos, Vos, Pirkis, & Carter, 2012).

**Depressive Symptoms**

Approximately 8% of all U. S. adolescents suffer from depressive symptoms (NAMI, 2013); however, these symptoms often go unnoticed. If early detection of depressive symptoms occurs in adolescence, interventions could begin to help with future symptoms or episodes of depression as the adolescent moves into adulthood (Abela &
Hankin, 2008). Research from the American Psychiatric Association (2013) also provides information that depressive symptoms have a high chance of recurrence. At least half of all individuals who recover from an episode of depression will have one or more episodes of depression in the future. Once an individual has a second episode, 80% of those individuals may have even more recurrences (Burcusa & Iacono, 2007).

NAMI (2013) provides specific examples of depressive symptoms in adolescents including: difficulty making decisions; fatigue and decreased energy; feelings of guilt, worthlessness, helplessness; hopelessness and/or pessimism; insomnia, early-morning wakefulness, or excessive sleeping; and a loss of interest in pleasurable activities or hobbies. In addition, other symptoms that may be more obvious to parents, teachers, or a school nurse may include overeating or appetite loss; persistent aches or pains; headaches, cramps, or digestive problems that do not ease even with treatment; persistent sadness, anxiousness; talk of suicide; or suicide attempts.

Adolescence is a common developmental period for depressive symptoms (Siegel, 2012). In the United States (U. S.), adolescents \( n = 9,863 \) in the 6th, 8th and 10th grade were assessed for self-report of depressive symptoms using two questions developed from the Diagnostic and Statistical Manual of Mental Health Disorders (DSM-I) (American Psychiatric Association, 2013). Researchers asked the adolescents if they had “felt sad, blue, down, or depressed almost every day for two or more consecutive weeks during the past 12 months,” and the second question was a series of yes or no questions related to what the adolescent might have experienced when they were feeling down during the last 12 months (Saluja, Iachan, Scheidt, Overpeck, Sun, & Giedd, 2004, p.
761). The investigators reported that 18% of adolescents had depressive symptoms with a higher percentage of females (25%) vs. males (10%) endorsing these items.

Allen, Moss, Giovino, Shopland, and Pierce (1992) examined persistent depressive symptoms in adolescents from the 1989 National Teenage Attitudes and Practice Survey (TAPSI) and found that 7.7% self-reported depressive symptoms. Out of that 7.7% who originally reported depressive symptoms (n = 9,315), there were 38.5% reporting continued depressive symptoms in a follow-up longitudinal study (Patten, Choi, Vickers, & Pierce, 2001). In a screening of rural adolescents in 9th, 10th, and 11th grade (n = 819), 13% self-reported depressive symptoms using the Reynolds and Kopak (1998), Adolescent Depression Scale. In this rural sample, depressive symptoms were related to gender as well as the positive and negative impact of life events (Puskar, Tusaie-Mumford, Sereika, & Lamb, 1999).

Catrett and Gualtney (2009) reported that adolescents who were surveyed in the United States National Longitudinal Study of Adolescent Health (ADD Health) found that depressive symptoms were associated with insomnia and risky behaviors in 7th-9th graders and 10th-11th graders. In a similar study, Braet, Van Vlierberghe, Vandevivere, Theuwis and Bosmans (2012), examined adolescents (n = 288) aged 12-18 in inpatient and outpatient settings and found an association between early memories, emotions, cognitive thoughts about oneself, one’s relationships to others (early maladaptive schemas), and depressive symptoms. Therefore, examining issues that cause stress in adolescents and screening for depressive symptoms is an important step in order to begin affecting change for the future of this population (National Institute of Mental Health, 2012-2014).
Stressful Life Events

A certain amount of stress is a necessary and healthy part of life; yet for some adolescents, increased amounts of stress may be related to mental health decline (Carlozzi et al., 2010; Goodman, McEwen, Dolan, Schafer-Kalkhoff & Adler, 2005). Selye (1956) defined stress as “an orchestrated set of bodily defenses against any form of noxious stimulus,” and he referred to this reaction as the General Adaptation Syndrome (p. 2). Holmes and Rahe (1967) linked stressful life events to both mental and physical illnesses in their adult patient population. In order to understand how individuals respond to stressors, it is also important to understand an individual’s perception of stress or the individual’s cognitive processes that take place between the encounter and the response (Lazarus & Folkman, 1984).

As a modification to the original Social Readjustment Scale developed by Holmes and Rahe (1967) to measure stressful life events, Coddington (1972) developed the Life Event Scale for Adolescents (CLES-A). This measure allows a quantitative assessment of the different adolescent life events occurring over the 12 months preceding administration of the survey. Braet et al. (2013) investigated stressful life events and depressive symptoms in adolescents and found a relationship between these two variables ($r = 0.69, p < 0.01$), particularly in late adolescence. Oldehinkel and Bouma (2011) examined depressive symptoms and stressful life events in adolescent males and females and identified females at higher risk of depressive symptoms particularly with regard to the sensitivity of the HPA axis. In an earlier longitudinal study, Tram and Cole (2000), examined stressful life events, depressive symptoms, and self-perceived competence in
9th grade adolescents \( (n = 468) \). Negative life events and lower levels of self-competence were found to be related to depressive symptoms \( (\beta = 0.20, R^2 = 0.27 \ p < 0.001) \).

**Perceived Stress**

Perceived stress has been defined as the degree to which situations in one’s life are appraised as stressful, uncontrollable, unpredictable, and overloaded (Cohen, Kamarck, & Mermelstein, 1983). Stress from a psychological perspective focuses on the individual’s evaluation or perception of an event or situation that could potentially cause harm. Once there is an interaction between the individual and the environment, psychological stress may occur as the person interprets the meaning of the interaction and considers the resources available for coping (Cohen, Kessler, & Gordon, 1997). An individual’s stress response is not totally based on the intensity or quality of the event but on the perception of the individual and contextual factors surrounding the situation (Cohen et al., 1983). When a person is under stress for an extended period of time, the result is a prolonged physiological response to stress, and many times an illness (physical or mental) may develop. Therefore, it is important to not only measure stressful life events themselves but to also measure an individual’s perception of stress (Cohen et al., 1983) and their physiological response (McEwen, 1998).

In a longitudinal study of adolescents \( (n = 352) \) from seven high schools in the U.S., parental and family support predicted the amount of perceived stress in this adolescent population (Weigel, Devereux, Leigh, & Ballard-Reisch, 1998). Researchers noted that perceived stress contributed to symptom patterns in adolescents aged 12 to 14 \( (n = 148) \), such as headaches, sleep disturbances, stomach aches, and poor appetite.
Social support was seen as a mediator of perceived stress in this study (Yarcheski & Mahon, 1999).

Bullying

In a 2011 national survey, researchers found that 28% of U. S. adolescents reported being bullied at school over the previous year (Robers, Zhang, Truman, & Snyder, 2012). Bullying is known to be associated with stress and depressive symptoms in adolescence (Swearer, Song, Cary, Eagle, & Mickelson, 2001; Van der Wal, De Wit, & Hirasing, 2003; Ybarra, 2004). In a meta-analysis of 28 longitudinal studies, researchers found that adolescents who were bullied doubled the risk of having problems with depressive symptoms over an average of seven years after the victimization takes place (Ttofi, Farrington, Losel & Loeber, 2011). Olweus (1994) defines bullying as a relational problem between peers when an individual is exposed to negative or aggressive actions repeatedly, overtime. Bullying occurs when power is exerted over an individual using physical/psychological strength, age, social status, or knowledge of vulnerabilities. The person who is bullied has difficulty defending him or herself, thus becoming helpless against the aggressor. Bullying may be seen in many forms including relational/social, physical, cultural, and cyberbullying (Hunt, Peters, & Rapee, 2012; Kowalski & Limber, 2013; Kubiszewski, Fontaine, Potard, & Auzoult, 2015; Stassen-Berger, 2007; Sumter, Baumgartner, Valkenburg, & Peter, 2012).

The Centers for Disease Control and Prevention Youth Behavior Risk Surveillance System (CDC, YRBSS) (2011) estimated that 20% of adolescents are bullied. Bullying may not occur on just one occasion but can be as often as several times
a week. Furthermore, bullying often occurs in places that adults or teachers may miss the bullying event such as school bathrooms, hallways, and outside of school settings (Lemstra, Nielsen, Rogers, Thompson, & Moraros, 2012).

When a bullying incident happens only once, the event can be seen as stressful and may create long term traumatic memories (Gower & Borowsky, 2013); however, bullying becomes chronic when it occurs more than once a month, and this type of ongoing stress is known to cause long-term problems both mentally and physically (Arseneault, Bowes, & Shakoor, 2010). Bullying has been associated with decreased self-esteem, loneliness, isolation, poor academic performance, missed school days, and a lack of peer relationships (Kaltiala-Heino, Fröjd, & Marttunen, 2010). Bullying was also found to be associated with a number of mental health issues such as anxiety, depressive and psychosomatic symptoms, and self-harming behaviors in adolescents (Bauman & Summers, 2009; Fisher et al., 2012).

Another word for bullying, peer victimization, is conceptualized as a chronic stressor that may have a negative impact on development of an adolescent that may lead to lower self-concept, lack of trust in others, avoidant behavior, and social withdrawal (Carney, 2008; Siegel, La Greca, & Harrison, 2009). In a meta–analysis of cross sectional studies related to bullying (peer victimization) and maladjustment (depression, loneliness, generalized and social anxiety, global and social self-worth), Hawker and Bolton (2000) found that bullying was strongly related to depression and less related to anxiety. In addition, Ybarra, Leaf, and Diener-West (2004) found that greater rates of self-reported depressive symptoms were found in relation to online harassment (cyberbullying) than in the absence of cyberbullying (OR: 3.38, CI: 1.78, 6.45).
Vaillancourt and colleagues (2008) reported that bullying has a physiological as well as a psychological impact, in that cortisol was dysregulated (increased or blunted/flattened effect) in adolescents who were bullied. Other researchers studying adolescents have reported bullying was found to be associated with cortisol hyposcretion (Ouellet-Morin et al., 2011), hypersecretion (Rudolph, Troop-Gordon, & Granger, 2010; Stroud et al., 2009; Vaillancourt et al., 2011), dysregulated diurnal rhythm, and blunted effect depending on the type and amount of bullying (Van den Bergh, Van Calster, Puissant, & Huffel, 2008).

While many adolescents are affected by bullying and depressive symptoms, 9th graders may be at greater risk (Mehta, Cornell, Fan, & Gregory, 2013). There are increasing vulnerabilities experienced by 9th graders as they adjust to high school, peer groups, and increased academic responsibility (Cushman, 2006; Forster et al., 2013; Wang, McDonald, Rubin, & Laursen, 2012). The 9th grade adolescent is undergoing physical, psychological, and social development (Blakemore & Robbins, 2012; Kar, Vijay, & Mishra, 2013; Steinberg, 2005), and the need for approval and success among this group of adolescents can be associated with depressive symptoms (Calvete & Cardeñoso, 2005; Yabko, Hokoda, & Ulloa, 2008). Landon and Preble (2008) examined bullying in fifth through twelfth grade adolescents and found that among males and minorities, 14-15 year olds reported significantly more bullying than other age groups. Other researchers have also reported an increased prevalence of bullying and depressive symptoms in 14 to 15 year olds as compared to older high school adolescents (Fredstrom, Adams, & Gilman, 2011; Schneider, O'Donnell, Stueve, & Coulter, 2012).
Cortisol

From a biological perspective, stress is considered the body’s way of fighting back in an effort to restore equilibrium, or allostasis (McEwen, 1998). When an individual perceives a situation as stressful, the hypothalamic-pituitary-adrenocortical (HPA) axis is activated, and cortisol (physiological biomarker) levels increase (McEwen, 2012; Von Werne Baes, De Carvalho, Tofoli, Martins, & Juruaena, 2012). Cortisol is a hormone that releases energy stores, enhances the immune system, and helps to increase learning and memory (Chrousos & Gold, 1992). McEwen and Seeman (1999) discuss that cortisol is one of the primary mediators related to the stress response that may have influence throughout the body. This influence may be positive or negative and may help to predict outcomes such as depressive symptoms (Wolkowitz, Burke, Epel, & Reus, 2009). Salivary samples may be used to measure cortisol as a non-invasive, cost-effective way to document changes in stress-related physiology (Granger, Johnson, Szanton, Out, & Schumann, 2012).

Herman et al. (2003) describes the first response to stress as essential to an individual’s health and survival. This response takes place in the sympathetic nervous system that causes epinephrine and norepinephrine to be released into the blood stream. The second response is a hormonal response that takes place in the hypothalamic-pituitary-adrenocortical (HPA) axis. Neurons of the paraventricular nucleus (PVN) of the hypothalamus secrete corticotrophin releasing hormone (CRH), which then signals the pituitary to release adrenocorticotropic hormone (ACTH). At this point, the adrenal glands synthesize and secrete glucocorticoids (cortisol). When the stressor ends, glucocorticoids work through negative feedback on the pituitary gland and other brain
regions including the hypothalamus, hippocampus, and prefrontal cortex. These actions stop any further production or release of CRH and ACTH (Herman et al., 2003).

Glucocorticoids (cortisol) are the final products of the HPA axis and have a circadian / diurnal rhythm. Normally, cortisol levels peak in the morning and steadily decline throughout the day with the lowest levels at approximately midnight. This process occurs through multiple interactions in the brain that coordinate homeostasis. Along with the diurnal rhythm there are short bursts of secretion of cortisol throughout the day. Another unique feature of cortisol is the cortisol awakening response (CAR). The CAR is superimposed on the diurnal rhythm and occurs approximately 20 to 30 minutes after awakening in the morning (Fries, Detterborn, & Kirschbaum, 2009). In stressful situations the cortisol feedback loop is “decoupled,” thereby generating a dysregulated cortisol secretion (high/low/blunted or flattened) (Charmandari, Achermann, Carel, Coder, & Chrousos, 2012; Nader, Chrousos, & Kino, 2010) and may trigger central nervous symptoms of depression (Djuric et al., 2008; Hammen, Henry, & Daley, 2000; Juster et al., 2011; McEwen, 2012; Palazidou, 2012).

It is important to recognize that adolescents may each have a different HPA axis response to stress. Cortisol has a well-established circadian/diurnal rhythm that changes throughout the day depending on stressors that occur and the unique diurnal rhythm for each individual. Pulsatile responses also that occurs periodically due to eating for digestion (Gunnar & Cheatham, 2003; Knutsson et al., 1997). Some individuals will show an increase in the level of cortisol when stressed and then return to baseline once the stress is over; others may react to the stressor and have a sustained stress response (Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001). While some
researchers will measure the response to stress by taking a sample of cortisol prior to an
induced stressful event and then after the stressful event (Gunlicks-Stoessel, Mufson,
Cullen & Klimes-Dougan, 2013; Kirschbaum, Wust & Hellhammer, 1992; MacMillian et
al, 2009; Marceau et al., 2014), the current study measured the cortisol diurnal rhythm or
change from morning to afternoon in an adolescent. Examining the relationships and
differences in morning and afternoon cortisol levels as well as the change from morning
to afternoon (diurnal rhythm or a negative change) is important when trying to
understand how stress affects an adolescent with regard to depressive symptoms
(McCabe, & Schneider, 2009; Shirtcliff, Armstrong, Slattery, Kalin, & Essex, 2011,
Smyth et al. 1997).

The adolescent brain has a heightened sensitivity to stress that may cause changes
in structure and function that may not be reversible, thereby, affecting the hippocampus,
prefrontal cortex, and amygdala (Giedd & Rapoport, 2010; Leussis & Andersen, 2008).
Specifically, Lopez-Duran, Kovacs, and George (2009) along with Guerry and Hastings
(2011) found dysregulated cortisol levels (cortisol levels that do not follow the normal
diurnal rhythm) in adolescents with depression as compared to those without depression.
Other studies provide evidence that HPA axis dysregulation precedes development of
depression and associated behaviors in adolescence and early childhood, and that
hypercortisolism is a predictor of mental health problems in an adolescent over the next
one to ten years (Adam, Doane, Zingarg, Mineka, Craske, & Griffith, 2010; Booij,
Bouma, de Jonge, Ormel, & Oldehinkel, 2012; Dietrich et al., 2013; Goodyer, Herbert, &
Tamplin, 2003; Janssens et al., 2012). Researchers have noted that prolonged stress leads
to prolonged exposure to cortisol, which may lead to poor health outcomes throughout
the lifespan, such as decreased cognitive function, decreased immune system functioning, and metabolic disorders (McEwen, 1998; McEwen, Eiland, Hunter, & Miller, 2012; Karatsoreos & McEwen, 2013).

Salivary cortisol is widely used in research with adolescents; however, there are few studies measuring an adolescent’s cortisol in school settings. Hazler and colleagues (2010) make an argument for the need of biobehavioral studies in field-based settings such as schools where adolescents are most commonly found. Studying the issue of depressive symptoms in adolescents in a school setting will provide a better understanding of the multiple interactions between psychological, sociological, behavioral, environmental, chemical, and neurological activities that occur both within and outside the adolescent. When working with adolescents, it is important to utilize a method of data collection that is appropriate and minimally disrupts the routine of classes and activities (Haavet, Dalen, & Straand, 2006). Therefore, this dissertation and pilot study were conducted in two high school settings.

Covariates

**Gender**

When considering stress and depressive symptoms in adolescents by gender, there are distinct differences. Adolescent females have a two to one greater prevalence of depressive symptoms compared to males once puberty is complete (Patton et al., 2008). Although this phenomenon is not completely understood, it is thought to be due to hormonal changes (estradiol) that may contribute to the sensitization of the brain with regard to stress (Hyde, Mexulis, & Abramson, 2008). In the adolescent male, testosterone
has been found to be associated with reduced hormonal stress responses (Romeo, 2013).
In a study by Calvete and Cardenoso (2005), female adolescents had lower levels of
positive thinking and scored higher on issues of negative problem orientation.
Adolescent females had a higher need for approval and success and were more likely to
suffer with self-focused negative thoughts with subsequent depressive symptoms. In the
same study, males scored higher on positive orientation and avoidance. Adolescent males
also showed more impulsivity, careless problem solving, delinquent behavior, and
justification of belief in violence. These researchers surmised that the cognitive
differences in adolescent males and females may explain the differences in depressive
symptoms and negative behaviors (Calvete & Cardenoso, 2005).

In another study, researchers found that adolescent females had higher levels of
depressive symptoms than adolescent males, especially those with mothers who had a
history of mood disorder (Garber et al., 2002). Similarly, Gyllenberg and colleagues
(2011) found that conduct and hyperkinetic problems in males predicted later
antidepressant use as compared to females with the same problems. Adolescent males
and females who lack social support from a significant other or special friend are also at
higher risk for with depressive symptoms (Risser, Cates, Rehman, & Risser et al., 2010).

Race

Race can be a factor that impacts depressive symptoms, particularly when
examined with regard to stressful life events. Individual races or ethnicities may
experience differences in stressors, resources for coping, and pubertal status maturity
(Cook, Doksum, Chen, Carle, & Algria, 2011; Hamlat, Strange, Abramson, & Alloy,
2013). Rohde, Beevers, Stice, and O’Neil (2009) found that one in five females met
criteria for minor depression, and those adolescents from minority groups were at especially high risk for minor depression.

Walsemann, Bell, and Maitra (2011) investigated school-level racial composition and how this affected depressive symptoms in high school students. This study was based on data from the U. S. National Longitudinal Study of Adolescent Health (ADD Health) (1994/1995) (Harris & Urody, 2008). Findings suggest that adolescents of minority race in a predominately White school may be at higher risk for depressive symptoms, and this may be related to discrimination, exclusion, or lack of school attachment (Walsemann et al., 2011).

In another study, Chen, Haas, Gillmore, and Kopak (2011) examined racial and ethnic differences related to depressive symptoms and found that Chinese-American males over the age of 15 had higher depressive symptoms than White males of the same age. Similarly, Lorenzo, Frost, and Reinhertz (2000) found that Asian-American adolescents had more depressive symptoms than White Americans, and these adolescents were more withdrawn and had more social problems.

Contrary to the above findings, Tummala-Narra (2014), found when comparing adolescents of an ethnic or racial minority who immigrated to the U. S. with U. S.-born adolescents, there were no differences in the degree of depressive symptoms. There were few studies that only examined depressive symptoms and race in adolescents, which indicates that many factors must be examined when looking at mental health, particularly gender, stress, environment, and family history of mental health problems (Beardslee, Gladstone, & O’Connor, 2012; Bertha & Balazs, 2013; Braet et al., 2012; Cummings & Druss, 2011; Garber et al., 2002; Hammen, 2009).
Socioeconomic Status

Disparities in physical and mental health are known to be correlated with low socioeconomic status, particularly with regard to racial and ethnic minorities (Cummings, 2014; Goodman Slap, & Huang, 2003; Shavers, 2007). Nebbitt and Lombe (2007) found a significant relationship between socioeconomic status and depressive symptoms particularly with African-American adolescent males living in public housing developments. In this study, depressive symptoms were higher for males than females and were explained as specifically being related to neighborhood characteristics. African-American adolescent males may be exposed to delinquent peers and can experience “fatalism” more so than African-American adolescent females who may be more closely monitored by parents and friends (Nebbitt & Lombe, 2007).

In a similar study, Mezuk and colleagues (2010) found that depression is significantly associated with socioeconomic status and is mediated by stress, particularly in African-Americans. Findings indicate that disadvantaged environments as well as poor psychological coping methods, poor infrastructure, and living under chronically stressful conditions contribute to this mental health outcome. In a study using the U. S. National Longitudinal Study of Adolescent Health (ADD Health), Goodman et al. (2003) found that socioeconomic status had an important influence on physical and mental health across the population. One third of depression was associated with lower household incomes and lower parental education. The results from this study provide support that socioeconomic status is critically important in studies with a focus on public health issues such as depressive symptoms in adolescents. Investigating adolescents and the environments where they grow and play may shed light on many of the behavioral,
physical, and psychological problems occurring in this population (Cummings, 2014; Goodman et al., 2003).

**Gender Identity**

In the current study, adolescents were asked about their gender identity and given the options of homosexual, bisexual, or unsure of gender identity. While the term sexual orientation is the appropriate term for individuals who are in the sexual minority, the school systems where the study was conducted preferred the word gender identity on the survey given to adolescents in the study. Investigators examining mental health issues in adolescents suggest that there is a significant increase in depressive symptoms among adolescents who are in the sexual orientation or gender identity minority otherwise known as “sexual minority” (King, et al., 2008; Martin, 2002; Marshal et al., 2011; Marshal et al., 2013; Uribe & Harbeck, 1992). However, in a study with adolescents ($n = 207$) who were seeking assistance for concerns about sexual orientation or gender identity, researchers found when compared to adolescents from the general population, that seeking help occurrences for depressive symptoms was similar among these groups of adolescents (Weber, 2009).

Researchers have explored the issue of depressive symptoms and bullying in adolescents with regard to gender identity (Marshal et al., 2011; Robinson, Espelage, & Rivers, 2013); both studies found that there is a significant relationship between sexual minority victimization, and depressive symptoms. In another recent study, researchers found that adolescents who were gender non-conforming were at higher risk of depressive symptoms. Bullying or maltreatment accounted for half of the increased prevalence of depressive symptoms in this study (Roberts, Rosario, Slopen, Calzo, &
Austin, 2013). While gender identity issues may not be the only contributor to depressive symptoms in an adolescent population, there is sufficient evidence to include this factor when examining depressive symptoms (Marshal et al., 2008; Marshal, 2013; Mereish, O’Cleirigh, & Bradford, 2014; Mustanski, Birkett, Greene, Hatzenbuehler, & Newcomb, 2014; Roberts et al., 2013; Robinson et al., 2013).

Pubertal Status

Peterson, Crockett, Richards and Boxer (1986) define puberty as a mark of adolescence. Puberty is a period when a child begins to develop adult features including the biological transition of reproductive physiology. During this period, endocrine changes occur related to gonadal hormones as well as changes in body shape, size, and development of secondary sexual characteristics. During the pubertal stage, there are changes in the individual’s neurobiology. Specifically, there is heightened sensitivity to cortisol and to social and emotional stimuli while the neural system is still maturing (Romeo & McEwen, 2006).

In a study examining 9, 11, 13, and 15 year old children and adolescents ($n = 82$), Gunnar et al. (2009) reported basal cortisol was highest in the 15 year old age group. These changes may reflect emotional and cognitive transitions and the amount of stress present at the time as compared to those in earlier adolescence. A score of 3 on a 1-4 point pubertal scale was associated with increasing basal cortisol levels (Gunnar et al., 2009). Puberty may not only affect cortisol levels but also affect regulation of emotion, attention, mood, and long-term goal-directed behaviors (Dahl & Gunnar, 2009).
Summary

Over the last twenty years, researchers have investigated depressive symptoms in adolescents and have recognized the importance of identifying this mental health issue (Kessler et al., 2001; Naicker, Galambos, Zeng, Senthilselvan, & Colman, 2013; Saluja et al., 2004; van Lang, Ferdinand, & Verhulst, 2007). However, little work has been done in school settings with regard to screening vulnerable adolescents such as 9th graders during their first year of high school. During this vulnerable period when the 9th grader is experiencing many physical, social, psychological, emotional, and cognitive changes, it is important for researchers and others working with 9th graders to ask questions about depressive symptoms (Dierker et al., 2001). As many as one in five adolescents will experience depressive symptoms at some point, with well over half of these adolescents having a recurrence within seven years (NAMI, 2014). A school setting is an appropriate place to identify adolescents who may be experiencing depressive symptoms (Sawyer, Borojevic, Ettridge, Spence, Sheffield, & Lynch, 2012).

Stress has a direct relationship to depressive symptoms in adolescent populations (Burghy et al., 2012), and bullying is a particular stressor that also has a direct relationship to depressive symptoms (Due, Damsgaard, Lund, & Holstein, 2009; Konishi & Hymel, 2009). Furthermore, there has been a plethora of studies over the years that indicate that there may be changes in the normal diurnal rhythm of the HPA axis or dysregulation when an adolescent has depressive symptoms (Burke, Davis, Otte, & Mohr, 2005; Foreman & Goodyer, 1988; Guerry & Hastings, 2011; Van den Bergh, & Van Calster, 2009).
Covariates to these relationships between stress, the HPA axis and depressive symptoms include gender (Brenning, Bosmans, Braet, & Theuwis, 2012; Felix & Mahon, 2006), race (Cummings & Druss, 2011; Kliewer, Dibble, Goodman, & Sullivan 2012; Walsemann, Bell, & Goosby, 2011), gender identity/sexual orientation (Mustansk et al., 2014; Roberts et al., 2013), socioeconomic status (Cummings, 2014; Lupien, King, Meaney & McEwen et al., 2001), and pubertal status (Gunner et al., 2009). Providing screening by asking questions about stressful life events, perceived stress, bullying, and depressive symptoms in the form of an online survey as well as collecting saliva for cortisol analysis in a school setting may provide an addition to the knowledge gap as to why depressive symptoms continue to be a problem in adolescence, particularly in 9th grade adolescents.

Purpose of the Study

The purpose of this study was to examine stressful life events, perceived stress, and bullying to determine the influence of these variables independently and cumulatively on depressive symptoms. In addition, cortisol (physiological biomarker of stress) was examined to determine if the cortisol diurnal rhythm has a mediating relationship between each of the independent variables (stressful life events, perceived stress, and bullying), and the dependent variable, depressive symptoms.

Statement of the Problem

Depressive symptoms among U. S. adolescents are a growing concern, as approximately 13% to 20% of children and adolescents are diagnosed with a mental
disorder in a given year (CDC, 2013). The U. S. Preventative Services Task Force (2013) is currently recommending routine screening for depressive symptoms in adolescent populations in primary care setting. In addition, Healthy People 2020 has a goal of reducing the proportion of persons who experience a major depressive episode and increasing the proportion of children and adolescents who receive treatment for mental health disorders (Department of Health and Human Services (HHS), Healthy People 2020, 2015). Carlson and Kashani (1988) found that when children and adolescents are depressed, they have similar symptoms as adults; however, there may be developmental differences. Recognizing depressive symptoms well before adulthood may lead to referrals for monitoring and intervention (Dumont & Olson, 2012; Hamlat et al., 2013; Humensky et al., 2010; Merikangas et al., 2010; Sawyer et al., 2012).

Stress and bullying are common experiences among adolescents and are known to be related to depressive symptoms (Burton, Marshal, Chisolm, Sucato, & Friedman, 2013; Dumont & Olson, 2012; Fredstrom et al., 2011; Schneider et al., 2012). If depressive symptoms are recognized early, this may help to prevent a lifelong trajectory of mental health concerns (Abela & Hankin, 2008; Bauman, Toomey, & Walker, 2013; Chen, Haas, Gillmore, & Kopak, 2011; Johnson, Riley, Granger, & Riis, 2013).

Stress is related to both physical and mental illnesses (McEwen, 2012). By measuring the physiological end product of the HPA axis (cortisol) in the form of the cortisol diurnal rhythm as well as examining the adolescent’s stressful life events, and their perception of these stressful events, this biobehavioral study may open doors to referrals for those adolescents who have depressive symptoms. The National Institute of Nursing Research (2011) strategic plan includes integrating biological and behavioral
sciences as a priority to improving the physical and mental health of individuals in the future. Previous researchers purport the feasibility of using biobehavioral methods to conduct research in “real world” settings such as schools (Granger, Johnson, Szanton, Out, & Schumann, 2012), and collecting saliva in a school setting is an economical, non-invasive way to measure cortisol in an adolescent population (Hazler et al., 2006; Kelly, Young, Sweeting, Fischer & West, 2008).

To date, there are no known studies examining stress (stressful life events, life change units, and perceived stress), bullying, cortisol, and depressive symptoms in a 9th grade population. However, several researchers have investigated peer victimization (bullying), child maltreatment, and other stressors in different age ranges from 8-17, and each found abnormal diurnal rhythms or a dysregulated HPA axis related to the stressor being examined (Rudolph et al., 2011). For example, Knack, Jensen-Campbell, and Baum (2011) examined peer victimization (bullying) and cortisol levels in (5th to 8th grade adolescents) and found not only dysregulated cortisol levels (flattened cortisol awakening response) but also poor physical health outcomes (more abdominal pain and frequent physician office visits). Ouellet-Morin and colleagues (2011) found that 12-year-old adolescents who had been bullied had lower cortisol responses in comparison to those adolescents who were not bullied. In addition, the bullied adolescents showed more social, emotional, and behavioral problems.

Rudolph, Troop, and Granger (2010) found that higher levels of cortisol were related to the frustration of bullying. Vaillancourt, Hymel, and McDougall (2013) discussed how bullying can have lasting effects after completing studies assessing bullying and cortisol levels showing a dysregulated HPA axis (flattening or blunting of
cortisol) as well as showing poorer memory and depressive symptoms among victims of bullying (Vaillancourt et al., 2008; Vaillancourt et al., 2011).

As the problem of bullying and other stressful life events in the U.S continues to increase, adolescents who are vulnerable to this type of victimization and stress can be assessed with traditional methods (surveys) in more naturalistic environments such as school settings while also using a biobehavioral method (salivary cortisol analysis) (Bauman, Toomey, & Walker, 2013; Bradshaw, Waasdorp, & O'Brennan, 2013; Casas, Del Rey, & Ortega-Ruiz, 2013; Ybarra, Boyd, Korchmaros, & Oppenheim, 2012). By assessing these variables of stressful life events, perceived stress, and bullying in this vulnerable 9th grade population, these adolescents who have depressive symptoms may be able to receive psychological counseling that might not have occurred if researchers were not asking questions about these matters (Cushman, 2006; Fredstrom et al., 2011; Mehta et al., 2013).

Significance of the Problem

Recognition of depressive symptoms or depression in adolescents was not a common occurrence before 1970; however, by the 1980’s, depressive symptoms that met criteria for major depression were documented in adolescents as well as children (Carlson & Cantwell, 1980). Today, mental health disorders are a leading cause of disability in the world, with 40.5% of the cases of mental health disorders being depression (World Health Organization, 2015). It is likely that most mental health problems, particularly depressive symptoms, first occur during the transition period of adolescence or between the ages of 13 and 18 years of age (Kessler, Berglund, Demler, Jin, Merikangas, &
Walkers, 2005; Merikangas et al., 2010). When mental health issues are not recognized and treated early, there may be long-term financial cost to the individual and to the U. S. Mental Health care system, with $532.1 million requested for the Mental Health Block Grant in 2015 (The National Council for Behavioral Health, 2015). Providing screening in an adolescent population is recommended by the Preventative Task Force in the 2014 report to the United States Congress of High-Priority Evidence Gaps (U. S. Preventative Task Force, 2014).

This study will provide an important avenue for early detection of depressive symptoms in 9th grade adolescents. There are no known studies that have examined 9th graders with a biobehavioral approach as they enter the high school setting. It is known that 9th graders are more vulnerable to stress (stressful life events, perceived stress) (Cushman, 2006). Considering the findings from the literature, attention should be given to stressful life events, perceived stress, and bullying that are more common in adolescence, particularly among 9th graders as compared to other high school adolescents (Hunter, Durkin, Heinn, How & Bergin, 2010; Lemstra et al., 2012; Schneider et al., 2012; Wang et al., 2012). If depressive symptoms are identified early in high school, the 9th grade adolescent may have a more successful life trajectory with regard to mental health.

Research Questions and Hypotheses

Research Question 1

What are the descriptive characteristics of the measures of stressful life events, perceived stress, cortisol diurnal rhythm, bullying and depressive symptoms in 9th grade adolescents?
Research Question 2

Are there correlations between stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms in 9th grade adolescents?

Hypothesis. There is a significant positive correlation between each of the variables (stressful life events, perceived stress, bullying, and cortisol diurnal rhythm) and depressive symptoms.

Research Question 3

Do stressful life events, perceived stress, bullying, and cortisol diurnal rhythm influence depressive symptoms in 9th grade adolescents after controlling for covariates of gender, race, socioeconomic status (SES), gender identity, and pubertal status?

Hypothesis. Each independent variable (stressful life events, perceived stress, cortisol diurnal rhythm, and bullying) will separately account for a percentage of the variance in depressive symptoms. Cumulatively, there will be a larger percentage of the variance in depressive symptoms explained by the combination of the three independent variables after controlling for gender, race, gender identity, socioeconomic status (SES), and pubertal status.

Research Question 4

Does cortisol diurnal rhythm mediate the relationship between stressful life events (SLE & LCU), and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity, and pubertal status?

Hypothesis. Cortisol diurnal rhythm mediates the relationship between stressful life events, and depressive symptoms after controlling for gender, race, gender identity, socioeconomic status, and pubertal status.
Research Question 5

Does cortisol diurnal rhythm mediate the relationship between perceived stress and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity and pubertal status?

Hypothesis. Cortisol diurnal rhythm mediates the relationship between perceived stress and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity, and pubertal status.

Research Question 6

Does cortisol diurnal rhythm mediate the relationship between bullying and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity, and pubertal status?

Hypothesis. Cortisol diurnal rhythm mediates the relationship between bullying and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity, and pubertal status.

Theoretical Framework

McEwen’s theory of Allostatic Load will be used as the theoretical framework for the current study (see Figure 1) (McEwen, 1998). Allostatic load theory purports that stressors affect an individual and his/her perception of stress. Allostasis is defined as a stable response to stress, and allostatic load is defined as the “wear and tear on the body and brain resulting from over-activity or inactivity of physiological systems normally involved in adaptation to environmental challenge” (McEwen, 1998, p. 37). Each individual has his or her own perception and response to a stressor such as bullying or
other events known to be associated with stress during an adolescent’s transition to high school (Cushman, 2006). Furthermore, each individual will have a unique physiological response (cortisol/HPA axis) and a unique cortisol diurnal rhythm when under stress that may lead to allostasis, allostatic load, or allostatic overload. Using McEwen’s Allostatic Load theory in describing why and how each individual responds to stressful life events, perceived stress, and bullying will help understand the relationship to depressive symptoms in 9th graders.

*Figure 1. McEwen’s Allostatic Load Theory*

In the last 20 years, the model of allostasis or allostatic load has been used to explain how psychological stress impacts an individual’s physiology. McEwen (1998) defined stress as an event that demands energy from an individual to cope with a challenge that is either physiological or emotional. The term “allostasis” was first coined in 1988 as meaning the body’s ability to stabilize as change or stress occurs (Sterling &
Eyer, 1988; McEwen, 1998). The brain is described as the organ that manages stress of all types (physical, social, and emotional) (McEwen & Gianaros, 2011).

Mediators are hormones such as cortisol and catecholamines that are released when a person is under stress. Other hormones including adrenocorticotropic hormone (ACTH), corticotrophin-releasing hormone (CRH), and arginine vasopressin (AVP), help with this stabilization process. These hormones are released into the pituitary portal circulation that drives the secretion of glucocorticoids (cortisol) (McEwen, 1997).

Although there are many hormones related to the stress response, the hormone cortisol and the individual cortisol diurnal rhythm was evaluated in the current study as a possible mediator of the relationship between stressful life events, perceived stress, bullying, and depressive symptoms in a 9th grade population. Cortisol can be measured in saliva, which is feasible to collect in a school setting (Hazler et al., 2006) and is also related to the other variables in this study (stressful life events, perceived stress, and bullying) (Adam et al., 2010; Booij et al., 2012; Burke et al., 2005; Gunnar et al., 2009).

When the stress response occurs due to issues in the environment beyond an individual’s control, there is an initial adaptive response (increased arousal, alertness, suppression of appetite, redirection of energy) that is necessary for survival. However, when the stress becomes chronic due to repeated exposure to the stressor, there may be a dysregulated response where the stress response fails to shut down even after the stressful event has passed. This dysregulated response is seen by examining the cortisol diurnal rhythm (McEwen, 1998; McEwen & Seeman, 1999).

During puberty, an adolescent’s nervous system may be more vulnerable to stress, as this is a period where certain areas of the brain (hippocampus, prefrontal cortex, and
amygdala) are continuing to mature (Romeo & McEwen, 2006). When an adolescent experiences a stressor, the adolescent’s immature brain experiences a different exposure to cortisol than an adult would experience. Thus, the brain experiences structural and functional “plasticity” where regions of the brain (hippocampus, prefrontal cortex, and amygdala) are also more sensitive to glucocorticoids (Dahl & Gunnar, 2009).

When an adult experiences stressors, there is evidence to show that the effects on the brain can be reversed; however, when stressors continue to occur during puberty, the results may last much longer or could cause permanent changes in the brain structure and function (Romeo & McEwen, 2006; Romeo, 2010). The perception of being accepted by one’s peers (social competence) may influence cortisol activity (Zwolinski, 2008). For example, cortisol reactivity was related to social information processing, particularly in females who report low relational victimization (Zwolinski, 2008).

Evidence indicates that a complex threat such as a social challenge require more integration of sensory information than previous experiences (Wang et al., 2012). This activity occurs in the higher limbic levels of the brain and may have an inhibitory influence on the HPA axis (Herman & Cullinan, 1997). Evidence indicates that adolescents’ exposure to stressors may lead to a greater chance of developing emotional or psychological issues such as depressive symptoms (Turner & Lloyd, 2004; Harkness, Stewart, & Wynne-Edwards, 2011; Holder & Blaustein, 2014).

Depression in adolescents is associated with an abnormal cortisol diurnal rhythm or dysregulation of the HPA axis. An imbalance of the glucocorticoid receptors (GR) and the mineralocorticoid receptors (MR) may initiate this dysregulation (Juster et al., 2011). The concept of allostatic load involves many aspects including environmental stressors,
individual differences in how a person responds to those stressors and the perception of the stress, a behavioral response, and ultimately a physiologic response that can lead to adaptation to the stressor (allostasis) or a decline resulting in either physical or mental illness (allostatic load or overload) (McEwen & Romeo, 2006). Therefore, this theory can be linked to the concepts of the current study, including stress (perceived stress and physiological response – cortisol), bullying, and depressive symptoms, and may lead to a better understanding of the pathophysiology between the stress response and depressive symptoms in adolescents.
Figure 2. Conceptual Framework

Conceptual Framework
Definition of Terms

_Adolescence_ – a period of transition in human development between puberty and adulthood involving physical, social, emotional, and psychological change (Benoit, Lacourse, & Claes, 2013).

_Depressive symptoms_ – difficulty making decisions, decreased energy, fatigue, feelings of guilt, worthlessness, helplessness, hopelessness or pessimism, insomnia, loss of interest in pleasurable activities, persistent sadness (National Alliance on Mental Illness, 2010-2015).

_Stressful life events_ – events that require a readjustment or a life change on the part of the individual (Coddington, 1972). The Coddington Life Event Survey – Adolescents (CLES-A) takes into account that the effect of a stressful life event may diminish over time; therefore, as participants mark the events occurring on the CLES-A, they must also mark whether the event occurred in the last 3 months (fully weighted), 6 months (3/4 weighted), 9 months (1/2 weighted) and year (1/4 weighted). There are 50 individual stressful life events on the CLES-A with scores (Life Change Units – LCU’s) ranging from 26 to 101 per event. The LCU score indicates the amount of social readjustment that is required by an adolescent. The actual number of stressful life events (SLE) was calculated as well as the score for the LCU.

_Perceived stress_ – “the degree to which situations in one’s life are appraised as stressful, unpredictable, unavoidable, or overloaded” (Cohen, Kamarck, & Mermelstein, 1983, p. 385).

_Bullying_ – a relational problem between peers in which an individual or group repeatedly exerts power over another individual through aggressive measures using size, strength
(physical and psychological), age, social status, or knowledge of vulnerabilities (Olweus, 2013).

Cortisol – a stress biomarker that is produced by the hypothalamic-pituitary-adrenocortical (HPA) axis, a physiologic system that governs neuroendocrine responses to stress (Slopen, McLaughlin, & Shonkoff, 2014).

Cortisol diurnal rhythm – the strongest cortisol activity is during the early morning hours for individuals who are normally active during the day. Peak cortisol levels are reached shortly after awakening with steady decrease in values (reflected as a negative number) in the absence of significant external stimulation. The trough of cortisol is reached around midnight with only minimal levels of cortisol detected (Anders, 1982).

Dysregulated cortisol diurnal rhythm - diurnal rhythm is flattened or blunted (less than 0.01 ug/dL change from morning to afternoon), or not following the expected cortisol diurnal rhythm (decline from morning to afternoon), an index of biological risks (Shirtcliff & Essex, 2009).

Assumptions

For purpose of this study, the following assumptions were made:

Adolescence is a period of physical, social, hormonal, and emotional change (Dahl & Gunnar, 2009).

Salivary cortisol levels can be measured in 9\textsuperscript{th} grade adolescents (Shirtcliff, Allison, Armstrong, Slattery, Kalin & Essex, 2011).
Summary

Researchers have identified the connections between stressful life events and depressive symptoms, perceived stress and depressive symptoms, bullying and depressive symptoms, and cortisol and depressive symptoms. However, in the adolescent population of 9th graders, no studies have been identified that examine the cortisol diurnal rhythm (change from morning to afternoon cortisol levels) and how this biomarker may be related to the interaction between stress, bullying, and depressive symptoms in 9th grade adolescents. Knowing more about this relationship will fill a gap in the information related to 9th grade adolescents and help those in settings where adolescents live, learn, and play to better recognize depressive symptoms and refer those adolescents for intervention.
CHAPTER 2
REVIEW OF THE LITERATURE

The purpose of this chapter is to review the literature that addresses the study variables stressful life events, perceived stress, cortisol diurnal rhythm, bullying, and depressive symptoms in 9\textsuperscript{th} grade adolescents. Each variable was examined within a time period of the last twenty years (1994–2014). Published research is presented about depressive symptoms, stressful life events, perceived stress, cortisol, and bullying. Each of these variables is discussed in the context of adolescence and the context of adolescents in the 9\textsuperscript{th} grade specifically. Finally, published research related to the confounding variables of gender, race, socioeconomic status, gender identity, and pubertal status were examined with regard to depressive symptoms.

A literature search was conducted using the following databases: CINAHL complete, Pub Med, and Scopus. In addition, other articles were found from reviewing reference lists of the articles identified in the search. Weekly notifications (2010-2015) were set up within the three data bases so that any new articles would be identified with any of the following key words: depressive symptoms, depression, anhedonia, adolescence, bullying, peer victimization, cortisol, HPA axis, stress, stressful life events, and perceived stress. A total of 26 articles containing at least three of the main study variables were found without duplications across the three search engines. Other seminal works and additional sources such as professional and advocacy organizations and government websites were reviewed to support this literature review.
Introduction

The U. S. spends approximately $135 billion to provide treatment for mental health and substance abuse conditions each year, which is 1.07% of the gross domestic product (Mark, Levit, Vandivort-Warren, Buck, & Coffey, 2011). There is evidence that stressful life events, perceived stress, and bullying precede depressive symptoms in adolescent populations (Juster et al., 2011; Kaltiala-Heino et al., 2010). Prolonged stress leads to prolonged exposure to cortisol and other hormones that can result in poor health outcomes such as decreased cognitive function, depressive symptoms, decreased immune system, and metabolic disorders (McEwen, 1998; McEwen et al., 2012; McEwen & Gianaros, 2011).

Many studies have examined these variables individually with different aged adolescents (Augustine et al., 2011; Dao et al., 2006; Glei, Goldman, Chuang, & Weinstein, 2007; Hamlat, Stange, Abramson, & Alloy, 2013). No evidence was found in the literature examining these issues together with regard to 9th graders. Examining depressive symptoms through the perspective of a 9th grader along with assessing stressful life events, perceived stress, bullying, and the physiological marker of stress (cortisol and cortisol diurnal rhythm) will add new knowledge to the literature that may help those working with 9th graders to better understand this mental health issue in a vulnerable population (Mehta et al., 2013).

Depression the United States

Before examining depressive symptoms in adolescents, the problem of depression in the United States must be recognized. The Centers for Disease Control and Prevention
(CDC) (2014) identified depression as a debilitating illness that causes suffering both mentally and financially often resulting in work absences, decreased productivity, or experiencing short term disability. The U. S. Preventative Services Task Force (2010) recommends that individuals age 18 and over be screened for depression and that efforts be put in place so that appropriate diagnosis and treatment can take place along with monitoring and follow-up. Previous research has found a positive relationship between depressive symptoms and stress in populations from early childhood to adulthood (Gold, Goodwin, & Chrousos, 1988a, 1988b; Hammen, 2006; Lee, Ogle, & Sapolsky, 2002; McEwen, 2005; Palazidou, 2012).

One in ten adults in the United States is known to report depressive symptoms (acute) (CDC, 2010). In the year 2012, an estimated 16 million adults over the age of 18 years had at least one major episode of depression, and 8.3% of the U. S. population had chronic depression (National Institute of Mental Health, 2012-2014). Furthermore, the World Health Organization (WHO) predicts major depression (chronic) to be the leading cause of disease burden by the year 2030 (WHO, 2004). Recognizing depressive symptoms earlier is important in order to prevent or decrease this trajectory of mental disability and to improve lives.

An understanding of depressive symptoms from a biological perspective is particularly important as many mental health problems in adults can be traced back to the adolescent age. Dahl and Gunnar (2009) call this the “key developmental window for understanding the emergence of psychopathology” (p.1). Adolescents who have depressive symptoms during puberty may go on to develop long term mental health problems (Braet, Vlierberghe, Vandevivere, Theuwis, & Bosmans, 2013; Burghy et al.,
2012; Dumont & Olson, 2012; Patton, et al., 2007). In the last several years, researchers have examined the biological marker of stress (cortisol) in adolescent populations in relation to depressive symptoms; however, there were inconclusive results (Carnegie et al., 2014; Chinthapalli, 2014; Doane et al., 2013; Essex et al., 2011; Gunlicks-Stoessel et al., 2013). Most of these studies were conducted in a clinic setting and all used different methods, measures, and age groups of students. The current study was conducted in a naturalistic (school) setting with a specific population of 9th grade adolescents.

Depressive Symptoms and Adolescents

Adolescence is considered a critical period for risk of depressive symptoms (Technow, Hazel, Abela, & Hankin, 2014). Costello, Erkanli, and Angold (2006) conducted a meta–analysis and found 26 studies examining depression in adolescents. Results of this study indicated that the depression prevalence rates were as follows: under age 13, 2.8%; 13 to 18 years of age, 5.6% in combined males and females; 13 to 18 year old females, 5.9%; and 13 to 18 year old males, 4.6%. Rates were found to be higher in adolescents than in children and higher in female adolescents than in male adolescents. Depression in adolescents has been linked to poor academic performance and poor interpersonal connections (Saluja et al., 2004) as well as a risk of suicidal thoughts or suicide (Lasgaard, Gossens & Elklit, 2011).

In the U.S., mental disorders among individuals younger than 24 years of age cost over $247 billion per year in terms of treatment costs, lost wages, and costs due to criminal acts (National Research Council and Institute of Medicine, 2009). At the present time, there are no routine screenings for depressive symptoms in primary care offices to
help identify adolescents at risk for depression so that referrals may be made to appropriate mental health providers. The U.S. Preventative Services Task Force’s (2014) recent report to the U.S. Congress recognized depression screening of adolescents as a high priority. Identifying adolescents at risk for depression may allow for referrals to appropriate mental health providers (Young, 2012).

The National Alliance on Mental Illness (NAMI) (2010-2015) reported that on any given day, approximately 8% of adolescents meet the criteria for major depression. Furthermore, the National Institute of Mental Health (2014) reports that 11% of adolescents will have a depressive disorder by the time they are 18 years old. Depression starts with depressive symptoms, which are recognized as the following: persistent sadness or blue, suicidal ideation or talk of suicide, irritability, deterioration in school or home functioning, complaints of physical ailments or making frequent visits to the school nurse, little interest in activities that previously brought pleasure, deterioration of relationships with friends, and abuse of substances (NAMI, 2011).

There are several national survey systems that measure depression or depressive symptoms among children and adolescents. Pratt, Brody, and Quiping (2011) gathered information from parents of children ages 4 to 17 and reported approximately 4.3% children ages 12 to 17 experienced depression for at least one two-week period in the years of 2005/2006. The National Health and Nutrition Examination Survey (NHANES) (2009-2012) used self-reported symptoms to identify depressive symptoms within the last two weeks among adolescents ages 12 to 17 and approximately 8% report current depressive symptoms. Rates of depression were seen in the National Survey of Children’s Health (NSCH) (2009/2010), which reported depressive symptoms among 2-17 year olds

After reviewing each of these national studies, there are many different ways of acquiring information about depressive symptoms in adolescents from a population perspective. None of these national studies have examined depressive symptoms from a physiological perspective, and the surveys are completed by various methods such as personal home visit, phone interview, a school based survey, or a computer survey. The rates of depressive symptoms vary greatly, and this may be due to the wide range of ages that are surveyed instead of limiting the surveys specifically to adolescents. Parents may not always be aware of depressive symptoms, or may pass these symptoms off as routine childhood or teenage issues; therefore, identifying depressive symptoms earlier may improve long term mental health outcomes (Hosseini, Rejali, Kheirabadi, & Aliyari, 2014).

Penden, Reed, and Rayens (2005) conducted a study with adolescents ages 14 to 18 ($n = 299$) in five rural high schools using the Center for Epidemiological Studies Depression Survey-20 (CESD-20). The prevalence for significant depressive symptoms (CESD-20 scores >16) in this population was 34%. The reports of depressive symptoms in males was similar to those reported by females, in contrast with other studies of depressive symptoms that showed that females reported more symptom than males (Mendle, Harden, Brooks-Gunn, & Graber, 2010; Paredes & Zumalde, 2014; Weeks et
These researchers also investigated stressful life events using the Life Event Checklist (Johnson & McCutcheson, 1980) and found that poor family relationships contributed to the depressive symptoms. Although this study provides insightful information, the results obtained in a rural setting may be considerably different than those obtained in urban or suburban studies considering the differences in population, socioeconomic status, and availability of treatment options.

Scott, Luxmore, Alexander, Tenn, and Christopher (2006) screened adolescents ages 13 – 19 (n = 192) during a visit to the pediatric emergency room (ER) and used the Beck Depression Inventory -2nd Edition (BDI-II). Approximately 25% of these adolescents presented with a mental health concern, and of these, 71% reported moderate to severe depressive symptoms. In this study, almost 1/3 of the total sample had either moderate or severe depressive symptoms regardless of their presenting concerns.

Adolescent depressive symptoms may be considered “subthreshold depression,” and are not always recognized (Fergusson, Horwood, Ridder, & Beutrais, 2005, p. 66). Limitations of this study were that the study was conducted in an emergency room where these adolescents were presenting for urgent health concerns. The adolescents may have been experiencing other problems such as pain, anxiety, or the presence of other patients or parents that could have made the adolescents participation in a depression screening an uncomfortable and less than truthful experience.

Chen, Haas, Gilmore, and Kopak (2011) conducted a secondary analysis examining depressive symptoms from adolescence to early adulthood over a period of 7 years using the U. S. National Longitudinal Study of Adolescent Health (ADD Health Study). Variables in this study included age, sex, race, ethnicity, socioeconomic status,
and acculturation. Depressive symptoms were measured with the CESD-20 but only used 9 of the 20 items, though the shortened version of the CESD-20 had not been validated. Results indicated that adolescents over age 15 had higher levels of depressive symptoms. Females reported more depressive symptoms than males. Chinese-American females had the highest CESD-20 scores among the two cultures. Other factors such as stress, family history, or physiological issues related to depressive symptoms were not examined and are considered a limitation of this study.

In the last ten years, studies have found that when an adolescent reports depressive symptoms, there may be recurrence in the adult years leading to significant mental health problems throughout the life course (Colman et al., 2014; Colman et al., 2011; Naicker, et al., 2013). Naicker and colleagues (2013) examined adolescents ages 16-17 (n =1,027) through the National Population Health Study in Canada. These researchers found that those adolescents who had depressive symptoms had significant effects 10 years later with depression recurrence and other problems such as migraines, poor self-rated health, and low social support. These researchers found that identifying depressive symptoms by screening in adolescence may mitigate problems in the future.

Depressive symptoms are considered a global health crisis as depression is a very common disorder (WHO, 2015). Since there is currently no standard screening of depression, individuals may suffer in silence due to the stigma of mental illness (Patel, 2013). Depressive symptoms are considered a precursor to mental health problems (Abela & Hankin, 2008). Given the early onset of mental health issues such as depressive symptoms, screening and outreach in schools makes sense (Merikangas et al., 2010). This
current study provides insight into what is happening physiologically in an adolescent with regard to depressive symptoms (Gunlicks–Stoessel et al., 2013).

**Depressive Symptoms among 9th Graders**

Depressive symptoms have been studied in the adolescent population in general; however, few studies have reported the prevalence of depressive symptoms in 9th graders. There is considerable evidence documenting the amount of stress 9th graders experience as they transition to high school (Chen & Gregory, 2009; Cushman, 2006; Hauser, Choate, & Thomas, 2009; Newman, Newman, Griffen, O'Connor, & Spas, 2007). Depressive symptoms in adolescents are seen in many studies as being related to stress (Braet et al., 2013; Brenning, Bosmans, Braet, & Theuwis, 2012; Hankin, Mermelstein, & Roesch, 2007). Understanding more specifics of the stress-depressive symptoms relationship in 9th graders is important; disseminating this information to those who work with 9th graders may lead to recognition of depressive symptoms and early intervention, thus, improving the adolescents’ quality of life.

Adolescents in 9th grade are typically 14 -16 years old, and this developmental period is considered middle adolescence. During the 9th grade period there are differences in biological, cognitive, and psychosocial development. Biologically, the 9th grader can be at varying levels of puberty (Foilb, Lui, & Romeo, 2011). These biological changes can be challenging for the 9th grade adolescent to understand and accept during a period of experiencing a new environment of high school with increased social opportunities, more freedom, yet increased difficulty in academics (Barone, Aquirre-Deandries, & Trickett, 1991; Bohnert, Aikins, & Arola, 2013; Hauser et al., 2010; Yeager et al., 2014).
Ninth graders are generally in a transition as they go from the “top to the heap” in 8th grade (middle school academic setting), to the “bottom of the heap” in 9th grade due to their move to the high school academic setting (Cushman, 2006). Beginning high school is a time where having friends or a peer group is important to bolster the adolescent who experiences an awkward period of hormonal changes, increased academic difficulty, and social/relational challenges (Anderman & Freeman, 2004; Newman, Myers, Newman, Lohman, & Smith, 2000; Newman et al., 2007). The “desire to belong” is basic to all human motivations; individuals need to have social relationships as a part of positive outcomes in everyday life (Baumeister & Leary, 1995, p. 499).

Ninth graders who have difficulty forming new peer relationships may experience more depressive symptoms than other 9th graders who feel connected to the school through activities and networks of friends (Langdon & Preble, 2008; Peskin et al., 2006). This lack of peer support may lead to feelings of loneliness, bullying, depressive symptoms, and possibly suicidal ideation or suicide (Bauman, Toomey, & Walker, 2013; Borowsky, Taliaferro, & McMorris, 2013; Gini & Pozzoli, 2009). Newman and colleagues (2007) found that 9th graders (n = 205) who self-reported more depressive symptoms also had lower levels of school belonging (r = -0.364, p < 0.001). Depressive symptoms were also significant when correlated with friend support (r = -0.153, p = 0.030), parental support (r = -0.389, p = 0.000), and stress (r = 0.198, 0.005).

Hankin, Badanes, Abela, and Watamura, (2010) examined 9th graders who were at risk for depression (n = 60) (dysphoric adolescents) as compared to 9th graders at low risk for depression (n = 223). This study reported higher cortisol of 9th graders who were dysphoric as compared to low risk adolescents (F (1.38, 114.53) = 3.29, p = 0.04). The
Changes that occur during the pubertal transition appear to provide important evidence in understanding the surge of depression seen from adolescence to adulthood.

Brenning and colleagues (2012) examined adolescents ($n = 228$) ages 12 to 18 to assess stressful life events, depressive symptoms, and cognitive schema vulnerability (negative belief regarding self and one’s relationship to others) and found that females reported more depressive symptoms than males ($F (1,226) = 20.38, p < 0.001$). In addition, Brenning et al. (2012) found that cognitive schema vulnerability was a partial mediator between life stress and depressive symptoms ($b = .06$ in the $4^{th}$ step of mediation process, $p < 0.05$).

Chen, Haas, Gillmore, and Kopak (2011) examined depressive symptoms in adolescents ($n = 20,745$) and found that among adolescents over the age of 15 years, depressive symptoms are less likely to be reported. Middle adolescents report a higher level of depressive symptoms than early or late-stage adolescents. Other findings from their study showed that females had higher levels of depressive symptoms than males in each age group (Chen et al., 2011). Researchers studied $9^{th}$ graders ($n = 632$) from five different high schools across the U. S. using the Center for Epidemiological Studies Depression Survey (CESD) and found similar results with females having higher rates of depressive symptoms than males ($Z = 2.5, p < 0.0006$) (Dierker et al., 2001).

Saluja and colleagues (2004) studied children ages 11, 13, and 15 ($n = 9,863$) in a school setting and found that 18% report depressive symptoms with a higher percentage of females (25%) as compared to males (10%), and as age increased, prevalence of depressive symptoms increased, particularly with females (three times more than males). Other predictors of depressive symptoms in this study included American Indian race and
adolescents who were bullied (Saluja et al., 2004). Sawyer and colleagues (2012) conducted a study with adolescents (n = 5,362) ages 12 to 14 who were experiencing depressive symptoms. These adolescents were asked about their intentions to seek help. Only 25% of adolescents with higher levels of depressive symptoms indicated that they were likely to seek help from anyone. These findings indicate the importance of screening for depressive symptoms in this population. If adolescents are not asked about these issues, the issues are likely to go unnoticed. Specifically, males are less likely to seek help for depressive symptoms (OR between 0.31 and 0.75) and less likely to seek help in general when compared to females (OR = 1.87).

Van den Bergh and Van Calster (2009) examined adolescents (n = 58) at 15 years of age for depressive symptoms and cortisol levels at three different times (awakening, noon, and evening) using the Children’s Depression Inventory (CDI). Adolescents who had higher levels of depressive symptoms (CDI > 13) also had a higher and flatter diurnal rhythm in cortisol with elevated evening levels of cortisol ($R^2 = 0.20$, $p = 0.001$) as compared with those with lower depressive symptoms (CDI < 13).

Stress in the United States

Stress is defined as “a state of real or perceived threat to homeostasis” (Charmandari et al., 2012, p. 1). The body responds to stress when an individual is challenged either externally or internally by a complex physiologic and behavioral adaptation (Charmandari et al., 2012). Another word for homeostasis is allostasis, and when the body fails to establish this balance, there is “dyshomeostasis” or “cacostasis”
(Chrousos, 2009, p. 374; Chrousos & Gold, 1992) or “allostatic load” (McEwen, 1998b, p. 171).

In the United States (U. S.) stress is becoming a public health crisis with more than 44% of Americans self-reporting an increase in stress in the last five years (The American Psychological Association, 2011). The American Institute of Stress reports that job stress is the most prevalent cause of stress with many Americans feeling they have little control over the future. Stress is a highly personal phenomenon that may be different for each individual depending on his/her unique situation (The American Institute on Stress, 2014). While stress has increased among U. S. adults, evidence indicates that stress is also a growing problem in adolescents (Hollenstein & Lougheed, 2013).

Stress may be viewed as both negative and positive in adolescents, and often linked to life events. Hobfoll, Freedy, Green, and Solomon (1996) reported the difference in extreme stressors as compared to less severe stressful life events. These events have properties identified as: (1) attacking a person’s most basic values (life and shelter), (2) making excessive demands, (3) occur without warning, (4) are outside of the realm of practical resources, and (5) leaving a powerful mental image (Hobfoll et al., 1996). Researchers suggest that stressful life events that precede the onset of depressive symptoms may cause changes in the neurobiological portion of the brain. As a result, the adolescent may be at higher risk of developing repeated depressive episodes (Gold, Goodwin, & Chrousos, 1988a, b; Sadock, Kaplan, & Sadock, 2007).
Stress in Adolescents

The adolescent period has been viewed as a transitional period of “storm and stress” (Hollenstein & Lougheed, 2013, p.44). Stress during adolescence has been reported to occur in psychological, biological, environmental, and emotional domains. Individual differences are associated with timing of puberty, temperament of the adolescent, typical transition into adulthood, and transactions of the adolescent within the world in which they live. Hamilton, et al., (2014) examined adolescents ($n = 318$) and reported that females who mature earlier than their female peers may experience a greater risk for depressive symptoms. In addition, stressors experienced during this period may exacerbate the emotional changes occurring and also increase vulnerability to mental disorders (Turner & Lloyd, 2004).

This study will examine stress from the perspectives of stressful life events and perceived stress. Two surveys included in this study are the Coddington Life Events Survey for Adolescents (CLES-A) (Coddington, 1972) and the Perceived Stress Scale – 10 (PSS-10) (Cohen et al., 1983). The CLES-A measures not only the stressful life event (SLE) but also the impact of the stressful life event or the life change unit (LCU). The following literature will discuss studies examining the variables of stressful life events, and perceived stress.

Stressful Life Events

Stressful life events have been found to play a primary role in the development of depressive symptoms (Garber, 2006; Garber, Weiss, & Shanley, 1993; Hammen, 2006; Rudolph, Flynn, & Abaied, 2008). Yet, little is known about how a stressful life event may interrupt normal neural development in an adolescent whose brain is still growing
and more vulnerable to mental health problems (Holder & Blaustein, 2014).

Developmentally, sources of stress are related to an adolescent’s age, gender, and development, particularly in the areas of physical growth, maturity level, and environmental influences (Berk, 2008). It is also important to understand stressful life events in relation to the maturation of the HPA axis, as changes in this process may alter an adolescent’s stress reactivity (Romeo, 2013; Romeo & McEwen, 2006).

Many different stressors are known to affect an adolescent; some of these stressors can be considered normative stress, which would be considered every day events such as fighting with parents over grades, peer relationships, or conflicts at school. Other stressful life events may be non-normative stressors, which are considered unusual or traumatic events such as a loss of a parent, a divorce of parents, or moving to a new school (Bronfenbrenner, 1986).

Duncan (1977) examined stressful life events in adolescents \( (n = 31) \) with regard to drug dependence. Adolescents who were dependent upon drugs reported higher life stress. Duncan’s (1977) study was limited in that there was a small sample size and only examined middle school/junior high school and senior high school adolescents. Hotaling, Atwell, and Linsling (1978) examined the relationship of stressful life events, social support and illness in college freshmen \( (n = 118) \). Parental support was found to mediate the relationship between social support and illness. High support group \( (n = 82, \gamma = 0.43; \chi^2 = 5.97, df = 2, p < 0.05) \) was compared to low support group \( (n = 36; \gamma = 0.62, \chi^2 = 8.71, df = 2, p < 0.01) \). In this study, stressful life events was measured using the CLES-A. Adolescents with a Life Change Unit (LCU) of 250 or greater reported
poor health, and there was a significant relationship between the number of stressful life events and illnesses ($r = 0.22$, $p < 0.01$).

Coddington and Troxell (1980) used the CLES-A for a study of high school football players ($n = 114$) to determine if players who reported more stressful life events had a greater risk of being injured while playing football. Player’s injury rates increased as much as five times when there was a threatened loss or when the actual loss of a parent occurred as compared to those who had no threatened or actual loss of a parent. These early studies indicate that stressful life events may contribute to some degree to physical illness as well as drug addiction, a form of mental illness.

Kaplan, Grossman, Landa, Shenker, and Weinhold (1986) examined stressful life events in relation to depressive symptoms in adolescents. The researchers categorized the adolescents into groups of acutely ill ($n = 43$), chronically ill ($n = 42$), and healthy controls ($n = 140$). Stressful life events were measured using the CLES-A and depressive symptoms were measured with the Beck’s Depression Inventory (BDI). A significant correlation was found between stressful/undesirable life events and depressive symptoms for those who were acutely ill ($r = 0.44$, $p < 0.01$) and the control group ($r = 0.43$, $p < 0.01$). In the chronically ill, there was a positive correlation between the Family Life Change score and the BDI ($r = 0.44$, $p < 0.01$) but not with the Undesirable Life Change score. Identifying stressful life events in adolescents can be helpful to healthcare professionals as they attempt to understand the trajectories of depressive symptoms.

Daniels and Moos (1990) examined life stress using the CLES-A and social resources (positive relationships with parents and peers) in association with depression in adolescents ($n = 90$). Adolescents who were depressed reported more life stressors (acute
and chronic), also reported fewer social resources. All of the above studies provide an evidence based foundation to the relationship between stressful life events, mental health, and physical health. However, none of these studies inquire about the adolescent’s perception of stress, nor do they provide a physiological measure of stress. Studies adding these measures may provide more specific evidence into the complex nature of this relationship.

The psychometric properties of the CLES-A have primarily been examined in white, middle class adolescents. More research is needed examining stressful life events in adolescents of diverse backgrounds (Rice, 2012). Furthermore, there are no specific studies which examine only the 9th grade experience of stress.

**Perceived stress**

It is important to measure an individual’s perception of stress as this can aid in determining if there is an increased risk for either physical or mental health disease (Cohen et al., 1983). The Perceived Stress Scale (PSS) measures an individual’s thoughts from a perspective of threat or demand with insufficient resources for coping (Cohen et al., 1983). Stress has been examined in several studies of adolescents with regard to various problems such as smoking, poor dietary habits, depression, and anxiety among many other physical and mental issues in adolescents (Austin, Smith, & Patterson, 2009; Hampel, & Petermann, 2006; Nguyen-Rodriquez, Chou, Unger, & Spruijt-Metz, 2008; Siqueira, Rolntizky, & Rickert, 2009).

The current study included the PSS-10, which is a ten-item questionnaire used to measure an adolescent’s perception of stress as it relates to depressive symptoms. There are several variations of the Perceived Stress Scale: the PSS-4, PSS-10, PSS-14, and PSS-
20. Reliability in the current study for the PSS-10 ($\alpha = 0.72$ to $0.85$ in previous studies) was better reliability than the PSS-4 ($\alpha = 0.64$), which was used for the pilot study. Different versions were developed to be appropriate for the population being studied and the amount of time needed to complete the PSS-10 (Cohen et al., 1983).

Austin, Smith, and Patterson (2009) conducted a two-stage study that examined stress and quality of diet in adolescents from a metropolitan area one month apart using the PSS-10 and the Daily Hassles Microsystem Scale. Quality of diet was measured with the Adolescents Food Habits Checklist. Data was collected at baseline and one month later. The investigators hypothesized that those adolescents who reported an increase in daily hassles over a one month period would report less-healthy eating during the time of stress. Males had higher perceived stress scores than females at stage one ($p < 0.05$) but no significant difference at stage two. Females showed a significant correlation with the change in diet and change in PSS-10 at stage two even when controlling for income level ($r = -0.697$, $p = 0.025$). These researchers found significant negative correlations between perceived stress and change in quality of diet.

Siqueira and colleagues (2000) examined stress in inner-city adolescents (12-21 years) ($n = 954$) in a clinic setting. These adolescent were either regular smokers, experimental smokers, and non-smokers. Stress was measured by the PSS-10, and stressful life events were measured by Norbeck’s (1984) Negative Life Event Scale (LES). Stress was higher in adolescent smokers compared to those adolescents who had quit smoking within the last six months. Smoking history was determined by questions such as “why did you continue to smoke after one or more attempts to quit smoking?” Most adolescents (83%) reported that stress was the most common reason for progressing
from being an experimenting smoker to smoking regularly. The Siqueira et al. (2000) study consistently indicated that those adolescents with higher perceived stress scores and more negative life events were less likely to quit smoking. Limitations of this study were that the PSS-10 does not provide a numeric cutoff point to provide guidance to the health care professional as to when to make referrals for counseling. In addition, the majority (82%) of participants were females and from an inner-city minority background, which limits generalizability.

In a randomly selected population based longitudinal cohort study, Hall, Moreau, Trussell, and Barber (2013) studied stress in females (n = 689) ages 18 to 20. The purpose of the study was to examine relationships between depression, stress, and consistency of contraception use during sexual activity using the PSS-4 and the CESD-5. Results indicated that when young women have elevated depressive symptoms as well has a higher perception of stress, there is a risk for inconsistent use of contraception. This study included a one-time sixty-minute in-person interview at baseline when females were asked about contraceptive methods and sexual history. In addition, participants completed weekly journals assessing reproductive, relationship, and health information over a 2.5 year period.

Hall and colleagues (2013) found that when females are stressed, they are less than half as likely to use contraception as compared to females with low stress. Limitations recognized were that psychological variables were only collected at baseline; therefore, these researchers were not able to evaluate time-variation relationships between psychological symptoms and use of contraceptives. In addition, this study was
not diagnostic of depressive symptoms, did not measure any physiological data, and can only be generalized to 18 to 20 year old females (Hall et al., 2013).

Zhang and colleagues (2014) examined perceived stress as related to social support, gender, coping, and depressive symptoms in adolescents aged 13 to 16 ($n = 1,674$) using the PSS–14 and CESD-13. When examining the multiple mediation model, results supported that the model fit indices were satisfactory for both males ($\chi^2(97) = 423.34, \ p < 0.001, \ CFI = 0.94, \ RMSEA = 0.06, \text{ and } AIC = 501.34$) and females ($\chi^2(97) = 350.72, \ p < 0.001, \ RMSEA = 0.06, \text{ and } AIC = 428.72$); however, the role of the family and other support mediated the relationship between perceived stress and depression with males but was not significant in females ($p > 0.05$). This was a cross-sectional design, which causes difficulty when determining casual relationships among variables. A helpful addition to this study may have been to collect data on stressful life events so as to better understand the participants’ perception of stress (Coddington, 1972).

**Stress in 9th graders**

Ninth grade is a usually a year where an adolescent transitions in the academic setting from middle school to high school (Cushman, 2006). During this time of development, social networks are readjusting, and adolescents begin to spend more time with peers and less time with family, all of which can be considered stressful in both positive and negative ways (Newman et al., 2007). The 9th grade period can be academically stressful in that evidence points to the fact that more adolescents fail 9th grade than any other grade in school. Adolescents of low socioeconomic status or minority are twice as likely to fail 9th grade. Many 14 and 15 year olds struggle with math and reading, and evidence indicates that 20% of these will drop out of school within two
years (Cauley & Jovanovich, 2006). Furthermore, many 9th grade adolescents begin high school feeling that the school will be huge and confusing, the academic work will be harder, and that as the youngest in the high school setting, they may be bullied by older adolescents. After being in school for a few weeks many 9th graders recognize that high school teachers have less individual time for students, and that teachers are not as forgiving when adolescents fail to complete assignments. Although high school often provides a fresh start in a new school setting, this transition may come with a new set of challenges. Developing a new identity, making new friends, and working hard academically can have long-lasting effects throughout high school and beyond (Cushman, 2006).

Newman and colleagues (2007) examined stressful life events, social support, school belonging, and depression in middle to high income, White (n = 205) 8th and 9th graders and measured stressful life events using the 39-item, Newcomb, Huba, and Bentler, (1981) Life Events Questionnaire. Researchers utilized data from 12 of the items from the Life Event’s Questionnaire related to parents and friends; school; personal health; appearance; interpersonal relationships; independence and freedom; and legal behavior. These questions each had a yes or no response, and adolescents were asked if the events had occurred in the last 12 months. Results indicated that after the move to a different school setting, 9th graders reported less social support and more depressive symptoms than 8th graders (Wilks lambda = 0.923. F (2,203) = 4.0, p < 0.01). Mean depression scores of 8th graders was 53.35 with SD = 12.96. Mean depression scores for 9th graders was 56.70, SD = 11.16. Stressful life events, gender, and grades in school were not significant predictors of depressive symptoms in statistical analysis (R² = 0.42, p
Peer support, parental support, and school belonging were significant when predicting depression \( (R^2 = 0.287, p < 0.05) \). In the Newman et al. (2007) study, the focus was on depressive symptoms and not specifically perceived stress or stressful life events. Another limitation noted was that only 12 of the 39 items from the Life Events Questionnaire were used, and this partial measure of life events has not been validated. Comparing perceived stress and stressful life events scores may have been helpful to have a better understanding of the stress related to the transition from 8th to 9th grade.

In a similar study, Barone, Aguirre-Deandreis, and Trickett (1991) measured stressful life events in 9th grade adolescents \((n = 113)\) using Swearingen and Cohen’s (1985) Junior High Life Experiences Survey (JHLES). There was a significant correlation between negative life events and Quality of School Life (QSL) \((r = -0.21, p < 0.05)\), meaning that 9th graders with higher life event scores were less satisfied with school and had more difficulty during the transition to a new environment of high school. In this same longitudinal study, Barone and colleagues (1991) found that the transition to 9th grade is associated with lower grade point average (GPA 2.58 to 2.19, \(t (114) = 6.92, 0.001\)) and lower school attendance (8.62 days to 10.28 days, \(t (114) = 1.90, p < 0.05\)). Furthermore, social support from friends and family may change, adding more sources of life stress than in previous years.

Hauser and colleagues (2009) conducted a study related to the transition to high school with adolescents. This study included adolescents \((n = 111)\), their parents \((n = 78)\), and their teachers \((n = 14)\). Findings from this study reported that 9th graders who have stronger social skills, are more outgoing, and are comfortable asking questions may
adjust more easily to a new high school setting (increases of over 15%). In this same study, 9th graders indicated that having friends or siblings at the high school, making a visit to the high school, and attending sporting events were helpful in making them feel part of the new environment (Hauser et al., 2009). In a study examining gender and race as variables in psychosocial adjustment in middle and high school, Akos and Galassi (2004) studied 6th grade adolescents (n = 173) and 9th grade adolescents (n = 320) and found that Latinos had a more difficult transition into high school than Caucasian or African-American adolescents (F (4, 160) = 4.54, p = 0.002). Although this study did not examine depressive symptoms specifically, the difficulty in transition may lead to depressive symptoms if not identified early in the process. In this case, school counselors, other adolescents, and family other than parents may be most helpful in this adjustment period (Akos & Galassi, 2010).

Larson and Ham (1993) examined 5th-9th graders (n = 483) using the CLES-A and found that the older adolescents reported significantly more negative life events than the younger pre-adolescents (F (16, 1,828) = 2.48, p < 0.001). These researchers also found that negative life events were a stronger predictor of negative affect in the older adolescents (R² = 0.10, p < 0.001) than the younger pre-adolescents (R² = 0.014, p > 0.05).

Some adolescents may experience high stress and continue to function normally against great odds (Masten, 2001). Adolescents who are more resilient to stressors or who have the ability to overcome may have a stronger cognitive ability; have a better ability to solve problems; consider stressors to represent an opportunity; have a positive view of self; a strong faith; have an optimistic, hopeful viewpoint; or have a sense of humor.
(Scudder, Sullivan, & Copeland-Linder, 2008; Thompkins & Schwartz, 2009). Other issues that may help to increase an adolescent’s resilience in the face of stressful life events may include close family relationships, higher socioeconomic status, parental involvement, positive parenting, and low levels of turbulence in the home (Scudder et al., 2008). While the current study does not examine resilience, this area is recognized as an area that could be added in the future.

The above findings concur with earlier studies where adolescents who underwent school change, puberty, and onset of dating relationships, had decreases in self-esteem, poor school performance, and less involvement in extracurricular activities (Simmons, Blyth, VanCleave, & Bush, 1979; Simmons, Burgeson, Carlton-Ford, & Blyth, 1987). Therefore, as the goal of the current study was to examine stressful life events, perceived stress, cortisol, and bullying in relation to depressive symptoms in 9th graders, this study will provide data specific to 9th graders and the difficulties faced when transitioning to high school.

**Cortisol**

The HPA axis is known as the system responsible for the physiological stress response. Cortisol has often been called the “stress hormone” (Akama & McEwen, 2005; Gunnar & Cheatham, 2003), but this hormone, otherwise known as a glucocorticoid is only one of many hormones released when an individual experiences stress (Johnson, Kamilaris, Chrousos, & Gold, 1992). The HPA axis is regulated by an area in the brain that includes the limbic system including the amygdala, hippocampus, and the prefrontal cortex. The hippocampus helps to regulate the activity of the HPA axis by a negative feedback system. This means that once a stressor occurs, the HPA axis is activated and
cortisol is released, once an appropriate level of cortisol is in the blood, and the stressor is no longer activating the HPA axis, the brain responds by decreasing the release of cortisol, thereby, allowing homeostasis to occur (Herman & Culliman, 1997; Herman et al., 2005; Jacobson & Sapolsky, 1991). Salivary cortisol is the biologically active form of cortisol, an unbound glucocorticoid that enters saliva via passive diffusion through the cells of the salivary glands (Vining, McGinley, Maksuytis, & Ho, 1983).

Cortisol has been evaluated by examining saliva since 1983 and has become the standard of measurement due to the non-invasive nature of saliva sampling (Kudielka et al., 2012). Cortisol follows a diurnal rhythm, meaning that levels peak in the morning and drop to the lowest levels at midnight. Cortisol levels are known to increase approximately 20 minutes after awakening, and this is referred to as the cortisol awakening response (Fries et al., 2009). Cortisol diurnal rhythm changes can have multiple effects on the body (Wolkowitz, Burke, Epel, & Reus, 2009). Some of these changes resulting from the “wear and tear or allostatic load” have been linked to diseases such as coronary artery disease, diabetes, obesity, depression, and cognitive impairment as well as inflammatory and or autoimmune disorders (McEwen, 1998a, p. 171). The current study examined the effects of the cortisol diurnal rhythm on depressive symptoms.

According to Kudielka and colleagues (2012), it may be difficult to determine “normal values” of cortisol as every individual is different. However, there is a normal range of cortisol in the adolescent age that is provided by Salimetrics, LLC that was utilized for this current study. When the cortisol diurnal rhythm does not follow the pattern as described by Anders (1982), this may be known as dysregulation of cortisol. When cortisol is higher than the normal range, this is considered as hyper (re)activity of
the HPA axis as seen in individuals who are chronically stressed or depressed. When cortisol is lower than the normal range, this is considered hypo (re)activity and has been seen in chronic fatigue syndrome, or traumatic events (Miller, Chen & Zhou, 2007). An abnormal diurnal rhythm can be seen as high and “flat” throughout the day, and this result is seen with internalizing symptoms such as depression or anxiety and is also seen when an adolescent is under chronic high stress (Klimes-Dougan et al., 2001). An abnormal diurnal rhythm can be seen as low or blunted morning levels and elevated levels in the evening can be seen when the individual has been exposed to trauma at an early age (Bevans, Cerbone, & Overstreet, 2008; Shirtcliff et al., 2011).

Romero (2013) reported that there are many changes that occur in the adolescent’s neuroendocrine system; however, the changes in the HPA axis are not as noticeable as the changes in the gonadal hormones related to puberty. Cortisol levels remain fairly constant through adolescence, but the amount and duration of hormones such as ACTH and glucocorticoids that are released in times of stress show a stronger response than in those of younger children (Stroud et al., 2009). Romero (2013) suggests that the changes in the adolescent stress response seem to involve both the activation and feedback of the HPA axis. Testosterone in males seems to reduce the stress response, and, in females, estrogen may increase response to stress (Viau, 2002). Adolescence is a period of time when there is continual neural growth and maturation. Excessive stress during this period of heightened sensitivity could possibly lead to stress-related behavioral changes during this period of important brain development (Romero, 2013).

Cortisol levels are known to vary in adolescent females, specifically for those who are taking birth control pills, who are pregnant, or who are breast feeding. Levels
may also vary depending on the menstrual cycle phase (Kudielka, Hellhammer & Wust, 2009), and these neurobiological changes are known to occur during puberty. Shirtcliff and colleagues (2011) examined children and adolescents \((n = 357)\) in 3\(^{rd}\) grade through 9\(^{th}\) grade and reported that as adolescents increased in age and physical development, their cortisol levels were lower \((\beta = -0.006, t (1037) = -2.25, p = 0.025)\) and flatter \((\beta = 0.0004, t (356) = 6.35, p < 0.0001)\) than those in the children in the study. In addition, findings indicated that females had higher cortisol with steeper slopes (change from morning to afternoon) \((\beta = 0.037, t (355) = 2.37, p < 0.018)\) than males \((\beta = 0.00011, t (9131) = 2.99, p < 0.003)\).

Depression has been associated with higher cortisol in daytime hours; however, studies are inconclusive as to the relationship with flatter as compared to steeper slopes of cortisol (Stetler & Miller, 2011; Van Den Bergh & Van Calster, 2009). Shirtcliff, and Essex (2008) studied 5\(^{th}\) – 7\(^{th}\) graders and found that high cortisol in 5\(^{th}\) grade put the adolescent at risk for mental health symptoms by 7\(^{th}\) grade. There was an overlap within individuals with regard to internalizing and externalizing symptoms in 5\(^{th}\) grade \((r = 0.45, p < 0.001)\) and in 7\(^{th}\) grade \((r = 0.47, p < 0.001)\). Having a flat diurnal rhythm was related to severity of symptoms. Shirtcliff et al., (2011) posits that cortisol is a “trait-like” hormone, particularly influencing the cortisol diurnal rhythm.

Puberty is also thought to be a period of time when there may be changes in the HPA axis (Dorn, Dahl, Woodward, & Brio, 2006), especially in adolescent females (Dahl, 1992; Keenan et al., 2013; Netherton, 2004; Tornage, 2002), as females are known to have higher cortisol than adolescent males (Rosmalen et al., 2005) and a steeper
cortisol diurnal rhythm with more curvature at each assessment (Schreiber et al., 2006; Shirtcliff & Essex, 2008).

Measurement of Cortisol

There are several ways to measure cortisol levels including saliva, blood, hair, and urine (Gallagher, Leitch, Massey, McAllister-Williams, & Young, 2006; Hamel et al., 2011; Inder, Dimeski, & Russell, 2012; Russell et al., 2012). Salivary cortisol has been used in research since 1983; however, little is known about how the HPA axis functions in a naturalistic setting (Kelly, et al., 2008). For the present study being conducted in a school setting, saliva collection was deemed the best method for measuring cortisol due to the noninvasive manner of collection (Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalkian, 2006; Hazler, Carney, & Granger, 2006; Kelly et al., 2008).

Researchers document that a wide range of factors must be considered for appropriate sample collection including: standardization of collection times, use of consistent materials and methods for collection, evaluation of recent food or drink intake, evaluation of medications and medical diagnoses, use of an appropriate assay and laboratory for processing, and establishing procedures and protocols for saliva collection (Hanrahan et al., 2006). Issues that may affect an individual’s salivary cortisol levels include the following: pregnancy, pubertal stage phase of menstrual cycle, smoking (Kirschbaum & Hellhammer, 1989; Kudielka, Gierens, Hellhammer, Wüst, & Schlotz, 2012), age, weight, gender, sleep time, and time of awakening (Kudielka, Broderick, & Kirschbaum, 2003), caffeine (Lovallo et al., 2005) elevated temperature, recent oral surgery or bleeding from gums (Kivlighan et al., 2004), and food intake just prior to
saliva collection (Granger et al., 2012). It is not feasible to screen individuals for everything that could affect cortisol, but having inclusion/exclusion criteria that include most of the major contributors that can cause changes in cortisol would be important when using cortisol as a biomarker (Kudielka et al., 2012).

Medications such as oral, nasal, topical, or ophthalmic anti-inflammatories, cholesterol-lowering medications; anti-ageing steroids; contraceptives; estrogens; anti-hypertensive medications; medications for glaucoma; chronic obstructive pulmonary disease; anti-cholinergic or medications for overactive bladder; irritable bowel disease; asthma or myasthenia gravis are known to affect salivary cortisol levels. When working with a population and testing cortisol levels, it is important to be mindful of participants who may be on any of these medications, as cortisol analysis may provide altered data for the researcher (Granger, Hibel, Fortunato, & Kapelewski, 2009).

Methods of collecting salivary cortisol should also be taken into account when studying this stress hormone. Hanrahan et al. (2006) proposes that with adolescents a passive drool saliva collection method is recommended. This procedure entails allowing saliva to pool in the mouth and then asking the adolescent to spit the drool into a short straw that is connected to a small collection tube (saliva collection device) (Granger et al., 2012). Other methods may include using a cotton dental roll or a cotton-tipped swab, depending on the age and cognitive ability of the individual (Harmon, Hibel, Rumyantseva, & Granger, 2007; Herrington, Olomu, & Geller, 2004).

Kudielka and colleagues (2012) described different study designs to use when incorporating salivary cortisol as a measure of stress including: day time cortisol levels, cortisol awakening response, event-related, time-based, or contextual within subject
designs. Granger and colleagues (2012) discussed other designs for measuring salivary cortisol including “basal level” or “steady state” levels, which are collected early morning before the events of the day occur (Booth, Granger, & Shirtcliff, 2008; Matias, Nicholson, & Friere, 2011); person-oriented approach, which are usually pre and post saliva sampling designed for measuring reactivity and recovery from a stressor; cortisol diurnal rhythm which typically measures four samples, one immediately after awakening, a second sample 30 minutes after awakening, a third sample at noon, a late afternoon sample, and a final sample before going to bed (Hellhammer, Wüst, & Kudielka, 2009).

For the current study, cortisol diurnal rhythm was measured by examining the difference between cortisol levels from a morning and an afternoon saliva sample. Other researchers have used this method in healthy children or adolescents (McCabe, & Schneider, 2009; Oskis, Clow, Thorn, Loveday, & Hucklebridge, 2012) and when examining issues such as puberty, emotions or mood (Adam, 2006), anti-social behavior (Susman et al., 2007), menstrual cycle (Wolfram, Bellingrath, & Kudielka, 2011) and temperament (Hankin et al., 2010).

When working with adolescents in a naturalistic setting, it is important to use a method of saliva collection that will accommodate the school day with few interruptions in academic courses and other important routines such as lunch, physical education, and testing situations (Granger & Kivlighan, 2003; Hazler et al., 2006). Collecting a morning and afternoon sample is feasible in a school setting, whereas other options of having adolescents or their parents assist in collecting their own specimens may result in inadequate or incorrect sampling methods (Granger et al., 2012; Granger, Johnson, Szanton, Out, & Schumann, 2012; Kelly et al., 2008).
Cortisol and Depressive Symptoms

Many studies have reported elevated cortisol associated with depressive symptoms (Bhagwagar, Hafizi, & Cowen, 2005; Burke et al., 2005; Fernald, Burke, & Gunnar, 2008; McCabe & Schneider, 2009; Miller, et al., 2007); however, most of these studies were done in adults. There are other studies that have reported an association between low cortisol levels and depressive symptoms (Bockting et al., 2012; Hsiao et al., 2010; Janssens et al., 2012; Juster, Smith, Ouellet, Sindi, & Lupien, 2013), and these studies are also primarily with the adult population.

Adam (2006) suggests that post-pubertal adolescents have been “understudied” with respect to cortisol diurnal rhythm. Lopez-Duran et al., (2009), conducted a meta-analysis of 17 adolescent studies examining the relationship of cortisol to depressive symptoms and found that basal cortisol levels were higher throughout the day in those who were depressed as compared to those who were not depressed. Van den Bergh and colleagues (2008) found that adolescents who had a flatter cortisol diurnal rhythm also had greater emotional distress. In either case, a high or low cortisol level would be considered dysregulation of cortisol, which plays a role in the precursors of depression (Von Werne Baes, De Carvalho Tofoli, Martins, & Juruena, 2012).

Guerry and Hastings (2011) posit that development of psychopathology consistently begins with the dysregulation of the HPA axis or an abnormal diurnal rhythm in relation to adolescent depression. Other researchers agree with this finding of cortisol dysregulation. With the ability to measure salivary cortisol using highly sensitive immunoassays, this form of research is feasible and can be adapted to work with broader populations including adolescents (Klimes-Dougan et al., 2001).
Doane and colleagues (2013) studied high school adolescents (n = 300) examining whether individual differences in life stress or negative emotion help account for altered HPA axis in adolescents without a diagnosis of depression. A racially diverse sample participated in a longitudinal study by providing saliva samples and diary entries six times per day for three consecutive days. Black adolescents reported more life stress over the last year as compared to White adolescents (F = 5.11, p < 0.05). Adolescents with past episodes of depressive symptoms had flatter cortisol slopes (r = 0.14, p < 0.05). Those adolescents with higher levels of chronic stress and episodic stress also had a past history of depressive symptoms. Limitations of this study were that the participants collected their own salivary cortisol samples in their home setting. The concern would be the fidelity of sample collection, storage, and return of the samples to the investigator. This research would suggest that changes in the HPA axis may be associated with internalizing disorders, such as depression. Feelings of sadness and loneliness may be considered the “active ingredient” in predicting alterations in cortisol (Doane et al., 2013 p. 639; Doane & Adam, 2010; Natias, Nicholson, & Friese, 2011).

It is not clear whether adolescent’s cortisol dysregulation makes him or her more vulnerable to depressive symptoms related to stress, or if the increase in stress disrupts the cortisol levels and then leads to depressive symptoms (Oldehinkel & Bouma, 2011; Owens et al., 2014). In either case, it is important to examine the issue of depressive symptoms in adolescents in a developmental manner, looking at both atypical and normal adolescent development (Cicchetti & Rogosch, 2002). Researchers have examined the HPA axis in adolescents as compared to other age groups of children and found higher basal cortisol levels and higher levels of cortisol stress response (Gunnar et al., 2009).
When an adolescent’s stress levels are high, and if the cortisol levels remain high over a period of time, this can lead to problems such as decreased immune system, lethargy, and changes in emotional functioning (Institute of Medicine, 2001). Adam, Sutton, Doane, & Mineka (2008) explain that the activation of the HPA axis begins in the limbic system or the emotional center of the brain. Adolescents are more sensitive emotionally due to this area of the brain maturing earlier than the prefrontal cortex, or the area that is related to cognitive development/thought processes (Adam et al., 2008; Stroud et al., 2009).

Gunlicks-Stoessal and colleagues (2013) conducted a pilot study with depressed adolescents ($n = 15$) who were not medicated but currently receiving interpersonal psychotherapy. The aim of the study was to determine the relationship of depression with cortisol. Investigators invited participants and parents to a laboratory setting for data collection. Salivary cortisol levels were first collected around 4pm, a time when cortisol should normally be lower (Kirschbaum & Hellhammer, 1989). Participants and their parents were provided a conflict scenario and asked to negotiate this conflict between them for approximately 15 minutes. Cortisol samples were again taken at 10, 20, and 30 minutes after the conflict to assess the cortisol response after a stressor. Samples of cortisol were also collected at 4, 8, 12, and 16 weeks after the initial visit to the laboratory. Cortisol levels should normally increase during interpersonal conflict (Kirschbaum et al., 1993); however, in this study, cortisol levels were decreased. Additionally, the severity of depressive symptoms predicted the levels of cortisol. In other words, adolescents with lower cortisol levels may be more withdrawn and have an intrapersonal style of coping where there is less activation of the HPA axis.
Although the above study had different purposes than the current study, the study used protocols in handling a broad range of race and age of adolescents involved for a pilot study. A limitation was that the study did not include adolescents without a diagnosis of depression so that those cortisol levels could be documented. Adding an age-matched population without depression would provide a comparison group to examine the cortisol responses to further elucidate the cortisol response in adolescents and the relationship to mental health (Hankin et al., 2010; Lopez-Duran et al., 2009).

The association of cortisol dysregulation or abnormal diurnal rhythm with depressive symptoms has been recognized by a number of researchers who study mental and physical health in adolescents (Burghy et al., 2012; Dieleman, van der Ende, Verhulst, & Huizink, 2010; Dietrich et al., 2012; Goodyer et al., 2003; Slopen, McLaughlin, & Shonkoff, 2014; Stewart, Mazurka, Bond, Wynne-Edwards, & Harkness, 2013). However, there is still a paucity of studies examining this relationship between dysregulated cortisol or abnormal diurnal rhythm and depressive symptoms in adolescents, especially 9th graders in a school setting. Guerry and Hastings (2011) suggest the importance of controlling for confounding issues that relate to stress from environmental and developmental contexts so that a better understanding is made available to those working with adolescents who may be experiencing depressive symptoms (McCabe & Schneider, 2009).

As in many studies, the cross-sectional, correlational data comes with limitations. Retrospective self-report may also be less reliable as would be longitudinal data (Gunnar et al., 2009; Oskis, Loveday, Hucklebridge, Thorn & Chow, 2009). Studies examining specific populations such as 9th graders who may be experiencing a stressful transitional
year as they start high school will add to the knowledge gap of the physiological and cognitive response to stress (both the stressful life events and the perception of stress) and how this relates to depressive symptoms. Examining cortisol both in the morning and afternoon shows a cortisol diurnal rhythm. This rhythm was examined in relation to depressive symptoms in the current 9th grade population.

Bullying and Adolescence

Bullying is defined as a relational problem between peers where power is exerted over an individual through aggressive measures using physical size, physical and psychological strength, age, social status, or knowledge of vulnerabilities (Olweus, 2003). One of the central concepts related to bullying is a power imbalance, which can be due to strength, size, age, socioeconomic status, or popularity at school (Schumann, Craig, & Rosu, 2013). Bullying has been conceptualized as a stressor as it has an impact on an adolescent’s development, which may decrease self-concept, inhibit trust in others, and may lead to withdrawal (Carney, 2008).

A national survey reported that 28% of U.S. adolescents reported being bullied (Robers et al., 2012). Bullying is recognized as a United States public health issue and can affect children as young as preschool and adolescents through the high school age (Centers for Disease Control and Prevention (CDC), 2014; Healthy People 2020, 2012; Schroeder et al., 2013). Williams and Guerra (2007) found that bullying peaked in 8th grade and began to decline in 11th grade, which adds to the importance of studying bullying in a 9th grade population.
Many studies are found in the recent literature related to many different types of adolescent bullying in the U.S., including physical, verbal/relational, cultural, and cyberbullying (Bogart et al., 2014; Caudle & Runyon, 2013; Desmet et al., 2014; Gini, Pozzoli, Lenzi, & Vieno, 2014; Hunt et al., 2012; Kowalski & Limber, 2013; Lapidot-Lefler & Dolev-Cohen, 2015; Low & Espelage, 2013). Studies have reported that bullying is perceived as a stressful event (Bjorkqvist, 2001; Hamilton, Newman, Delville, & Delville, 2008; Knack et al., 2011; Newman, Holden, & Delville, 2005).

An adolescent may be bullied for many reasons including but not limited to: younger age, gender (Scheithauer, Hayer, Petermann, & Jugert, 2006; Smith, Madsen, & Moody, 1999), lower SES (Peskin et al., 2006; Pickett et al., 2013; Viner et al., 2012), and social exclusion (Jennifer, 2008; Lindsay & McPherson, 2012; Thornberg & Knutsen, 2011). Bullying by electronic means may have other associated links that are very different from the reasons linked to traditional forms of bullying (physical, verbal, relational) such as gender identity or sexual orientation differences (Collier, van Beusekom, Bos, & Sandfort, 2013; Friedman, Koeske, Silvestre, Korr, & Sites, 2006; Gini & Pozzoli, 2009), and racial or cultural differences (Bachman, Randolph, & Brown, 2011; Goldweber, Waasdorp, & Bradshaw, 2013; Pozzoli, Ang, & Gini, 2012). With regard to cyber or electronic bullying, most individuals know the perpetrator who is committing the electronic victimization; yet, the psychosocial damage from cyberbullying can be a precursor to depressive symptoms (Feinstein, Bhatia, & Davila, 2014).

In an online study with college students (n = 565), victims of cyberbullying reported increases in depressive symptoms (Feinstein et al., 2014). Cyberbullying is
defined as victimization occurring via the internet, cellphones, electronic media, text messaging, or instant messaging (Kowalski & Limber, 2013). Recent estimates of the prevalence of cyberbullying prevalence range from 4% to 39% in adolescents (Adams, 2010; Bauman, Cross, & Walker, 2013; Brighi, Guarini, Melotti, Galli, & Genta, 2012). Studies have indicated a strong association between depressive symptoms and cyberbullying in children and adolescents (Rossi, Bernardo, Sterpa, & Ottolini, 2012; Schneider, O'Donnell, Stueve, & Coulter, 2012; Van Geel, Vedder, & Tanilon, 2014).

Bullying and 9th Graders

The Youth Risk Behavior Surveillance Study (YRBSS) (2013), a national study, reported that approximately 20% of adolescents were bullied on school property with higher prevalence of bullying reported by females than males. The YRBSS (2013) also reports that approximately 15% of adolescents reporting being cyberbullied with higher prevalence in females compared to males. In a study examining bullying in 9th grade adolescents, researchers found that adolescents who were more committed to school and school activities were less likely to perceive a climate of bullying after controlling for confounders of gender, race, school size, proportion of minority adolescents in the school, and individual level of perception of bullying (Mehta et al., 2013).

Rosen, Beron, and Underwood (2013) found that from 7th to 9th grade, social bullying increases significantly, especially in girls. Another study by Langdon and Preble, (2008) conducted with 5th – 12th graders (n = 3,147) found a positive correlation between lack of peer respect from peers (r (2,421) = 0.39) and adults (r (2,418) = 0.24), particularly in 9th graders. This lack of respect was positively correlated with more bullying (Langdon & Preble, 2008). In addition, males (t = 3.05, p = 0.002), minorities (t
= 2.22, \( p = 0.03 \)), and non-college-bound adolescents had higher rates of bullying, tended to be “different” (“losers, special education, poor hygiene, wrong clothes, not popular, LGBT, or non-athletic” p. 493-494) \( (F = 6.12, p = 0.002) \). This study also found that bullying often takes place out of sight from teachers in areas such as bathrooms, locker rooms, and on the bus (Langdon & Preble, 2008).

Cornell, Gregory, Huang, and Fan (2013) investigated 9th graders \( (n = 7,082) \) to determine whether bullying was predictive of dropping out of high school in a four year period. This prediction was found to be true in that one standard deviation increase in adolescent teasing and bullying self-reports predicted a 20.6% increase in the adolescent dropout rate, which translated to a 10.2% drop out rate in freshmen adolescents. Although many other factors may be associated with dropout rates in high school such as decreased school engagement, poor academic performance, and inattentiveness at school (Juvonen, Wang, & Espinoza, 2011), bullying could lead to the above problems and should be considered a risk factor.

Ryoo, Wang, and Swearer (2014) examined 5th – 9th graders \( (n = 1,180) \) in a longitudinal study over three time points, finding instability in bullying perpetration and victimization over time, especially during the transitional years (5th to 6th grade) and (8th to 9th grade). While results indicated that bullying victimization was less in 9th graders (6%) than in 5th graders (21%), 9th graders reported a higher probability of being a victim of occasional cyber and traditional bullying (26%) compared to other grades (4% to 13%). Ninth graders also reported fewer perpetrations as they began their high school years.
There is a plethora of recent studies in the global literature about bullying and depressive symptoms in adolescents (Nansel et al., 2001; Tam & Zhang, 2012). Using the Environmental Risk (E-Risk) Longitudinal Twin data base from England and Wales, Arseneault, Milne, Taylor, Adams, Delgado, Caspi and Moffitt (2008) found that when a twin was victimized by bullying, at 10 years of age, there was a significant difference in internalizing symptoms (depression or anxiety) when compared to the twin who had not been bullied ($t_{1,112} = 3.80, p < 0.001$).

In addition, Arseneault and colleagues (2008) found that being bullied has an environmentally mediated effect on children’s anxiety and depressive symptoms (internalizing problems) ($\beta = 0.36 [95\% CI, 0.18 – 0.54], p < 0.001$). The effect was still significant after controlling for any existing symptoms of depression or anxiety ($\beta = 0.26 [95\% CI, 0.09 – 0.44], p < 0.001$). Similarly, Bauman and colleagues (2013) examined the YRBSS survey and found that traditional bullying/victimization was a significant predictor of depression for both males ($\beta = 0.20, p < 0.001$) and females ($\beta = 0.13, p < 0.01$). In addition, depression was found to have a mediating effect between traditional victimization and suicide for both males ($a\beta = 0.13, 95\% CI = 0.06-0.21, p < 0.001$) and females ($a\beta = .11, 95\% CI = 0.04 - 0.31, p < 0.001$). Traditional bullying would be considered bullying by physical, verbal, relational, and indirect methods (Rigsby, 2007).

Borowsky, Taliferro and McMorris, (2013) conducted research with the 2010 Minnesota Student survey to examine suicidal thinking and behavior in those 6th, 9th, and 12th graders ($n = 130,908$) who experienced verbal and social bullying. These researchers found a significant difference between those adolescents involved in bullying as compared to those not involved in bullying ($\chi^2 = 6,509.8, p < 0.0001$). In this study,
females were significantly more likely to report suicidal thinking or behavior ($\chi^2 = 58.9, p < 0.001$). In addition, suicidality peaked in the 9th graders as compared to the 6th and 12th graders ($\chi^2 = 163.8, p < 0.001$).

Dao and colleagues (2006) studied 7th grade adolescents ($n = 186$) in North Florida to determine prevalence rate of bullying victimization and found that 14.6% experienced some form of aggressive victimization in the last month. In addition perceived risk of victimization (PRV) was mediated by the effect of past experience of victimization (PEV) on non-specific psychological distress (NSPD) or symptoms of depression and anxiety. These researchers used Baron and Kenny’s (1986) mediation criteria first finding that there was a significant relationship between PEV and NSPD ($\beta = 0.32, p < 0.001$), then finding a positive effect of PEV on PRV ($p < 0.05$). The third step was finding a significant relationship between PRV and NSPD ($\beta = 0.30, p < 0.001$). Finally finding a significant reduction in strength as the PEV was no longer significant when PRV was entered into the analysis ($R^2$ was .32 at $p < 0.05$ [$F(3,184) = 7.28$, $p < 0.001$], and PEV accounted for the most explained variance at ($R^2$ change = 0.17), followed by PRV ($R^2$ change = 0.10), and gender ($R^2$ change = 0.05). Three surveys were used in this study including: the Victimization Experience Survey (VES), the Risk of Victimization Survey (RVS), and the Perceived Risk of Victimization Survey (PRV).

Other studies report similar findings from other countries including a study from Due and colleagues (2009) that examined the Youth Cohort of the Danish Longitudinal Health Behavior Survey (DLHBS) and found that low childhood socioeconomic status ($p = 0.03$), exposure to bullying ($p = 0.002$), and being a female ($p = 0.002$) significantly increased the risk of depression as a young adult. Fekkes, Pijpers, and Verloove-
Vanhorick, (2004) studied 9 to 12 year old Dutch children (n = 2,766) and found that victims of bullying had higher odds of depression (OR: 7.7) as compared to those who had not been victimized by bullies. Fleming and Jacobsen (2009) found that bullying is common (47%) in middle school adolescents (n = 8131) in Chile, and 30% of these adolescents reporting depressive symptoms. Interestingly, 9th grade adolescents reported higher levels of loneliness, difficulty sleeping, and suicidal thoughts than the 7th and 8th grade adolescents (p < 0.001).

Gladstone, Parker, and Malhi (2006) examined males and females (n = 222) from Australia to determine if being bullied as a child results in anxiety or depression as an adult. Results showed that 31.5% were exposed to bullying as a child, and those who were bullied as a child had significantly higher scores on the Beck Depression Inventory (F = 11.1, df = 1,197, p < 0.001) and distinctly more symptoms of current anxiety (F = 4.9, df = 1,197, p = 0.028) as compared to the non-bullied participants. Iyer, Dougall, and Jensen-Campbell (2013) found when examining adolescents (n = 157) that bullying was significantly related to both depression (r = 0.50, p < 0.01) and anxiety (r = 0.22, p < 0.01).

Nadeem and Graham (2005) examined Black and Latino 6th grade adolescents (n = 1,024) and found that early maturing males and females who were viewed a victims of bullying reported increased depressed mood (females, r = 0.37, males, r = 0.33), more physical symptoms (females, r = 0.33, males, r = 0.24, both p < 0.001), and lower self-worth (females, r = -0.45, males, r = -0.44, both p < 0.001) than their peers who were not identifies as victims of bullying.
There are not as many studies that include the variables of bullying, depressive symptoms, and the physiological marker, cortisol. All of the following studies have been discussed in other areas of this dissertation as related to specific variables: Hamilton et al., 2014; Newman, Delville & Delville, 2008; Kliwer et al., 2012; Masten, et al., 2011; Ouellet-Morin et al., 2011; Knack et al., 2011; Rudolph et al., 2010; Stroud et al., 2011; Vaillancourt et al., 2008; Vaillancourt et al., 2011. No known studies were identified that examined the combination of variables in this study (stressful life events, perceived stress, cortisol diurnal rhythm, bullying, and depressive symptoms) in a 9th grade population.

Potential Confounding Variables

When examining stressful life events, perceived stress, cortisol diurnal rhythm, bullying, and depressive symptoms in 9th grade adolescents, many confounding variables can influence these predictors as well as the outcome variable of depressive symptoms. The confounding variables examined for this study include gender, race, socioeconomic status, gender identity, and pubertal status.

Gender

Evidence indicates that there are differences in depressive symptoms between male and female adolescents with females normally experiencing depressive symptoms at higher levels than males (Mendle et al., 2010; Paredes & Zumalde, 2014; Weeks et al., 2014). Hankin et al. (1998) discuss by the age of 15 years, the prevalence of depressive symptoms or depression in females is about two times higher than in males. As of 2012,
the Substance Abuse and Mental Health Services Administration (SAMSHA) reported that the prevalence of females ages 12 and 15 who experience major episodes of depressive symptoms have tripled (5.1% to 15.2%). As many as 1.4 million adolescent females have depressive symptoms as compared to adolescent males in the U. S. (Substance Abuse and Mental Health Administration, SAMHSA, 2012). There is also evidence that adolescent females may experience more stress that can lead to depressive symptoms than males (Hankin et al., 2007).

In other studies, depressive symptoms were seen in adolescents, both males and females, who experienced earlier puberty than their peers (Ge & Natsuaki, 2009; Negriff, Fung, & Trickett, 2008). Other researchers posit that the depressive symptoms in either males or females are more related to stressful life events rather than the gender alone (Hamilton et al., 2014; Rudolph & Troop-Gordon, 2010; Sontag, Graber, & Clemans, 2011).

Raffaelli and colleagues (2013) examined gender and age related differences in Mexican adolescents ($n = 5,152$, ages 17 – 20); significantly more females (28%) reported depressive symptoms than males (18%) ($p < 0.001$). In addition, this study found that when females are reporting depressive symptoms, they are 82% more likely to smoke and 20% less likely to exercise than those peers that were non-depressed. Adolescent males showing depressive symptoms were 30% more likely to drink than non-depressed males (Raffaelli et al., 2013).

Adolescent females have been known to exhibit relational aggression tendencies, for example: manipulating relationships to harm others or threatening to withdraw from a relationship (Crick & Grotpeter, 1996). Adolescent males on the other hand have been
characterized as more aggressive in a physical sense (Moffitt, 2001). However, other researchers found very few differences between the genders related to aggressive relationship behaviors (Kuppens, Grietens, Onghena, Michiels, & Subramanian, 2008). There is evidence that group exclusion and relationship aggression is harmful to adolescent males (Swearer, Turner, Givens, & Pollack, 2008); however, adolescent females may also show behaviors of physical aggression (Prothrow-Stith & Spivak, 2005). Goldweber and colleagues (2013) found that adolescent females were 1.4 times more likely to be characterized as victims, and adolescent males were more likely to be characterized as bully-victims.

In a different study examining femininity, relational and social aggression, and depressive symptoms, Kolbert, Field, Crothers, and Schreiber (2010) found that adolescent females who used relational and social aggression toward other females in late adolescence had higher levels of depressive symptoms. These researchers speculated that this could be related to the perception that females “should not” show aggressive tendencies in a physical manner. Depressive symptoms can also occur more frequently during a transitional period such as the move from 8th to 9th grade in high school. Conway, Rancourt, Adelman, Burk, and Prinstein (2011) found that this particularly affects adolescent females even as young as 6th grade when they are on the periphery of friendship groups.

Recognizing gender differences with regard to depressive symptoms is important; particularly in those who may be in transition to high school, as this may be a time of heightened interpersonal risk (Bi, Ma, Yuan, & Zhang, 2015; Conway et al, 2011). Understanding the dynamic changes that occur both physically and cognitively in
adolescent males and females in the 9th grade will further help to clarify why these differences may occur.

Race

Race has been associated with the outcome variable of depressive symptoms, as well as with the other independent variables in the current study such as stressful life events, perceived stress, bullying, and pubertal status in adolescents. For the purposes of this study, African-Americans were described as Black, and Caucasian Americans were described as White. Nadeem and Graham (2005) examined peer victimization/bullying, physical symptoms, self worth, and depressed mood in Black and Latino sixth graders (n = 1,024). Results indicated that Black and Latino males and females who have been peer-victimized self-reported more episodes of elevated depressed mood (r = 0.37, females, r = 0.33, males,) an increase in physical symptoms, (r = 0.33, females, r = 0.25, males, p < 0.001) and lower self-worth (r = -0.45, females, r = -0.44, p < 0.001) than their peers who were not victimized. Self-reported victimization predicted lower self-esteem, depressed mood, and more physical symptoms (β = 0.26 to 0.41, p < 0.001). Early maturing females and self-report of peer victimization/bullying were found to have the highest depressive symptoms scores. In a similar study examining perceived pubertal timing, externalizing behaviors and depressive symptoms in 15 year old Black-American and Caribbean-Black females (n = 607), Carter, Caldwell, Matusko, Antonucci, and Jackson, (2011) did not find a significant relationship between ethnicity and depressive symptoms.

When comparing Black females to White females ages 6 to 15, DeRose, Shiyko, Foster, and Brooks-Gunn (2011) found that early developing White females report more
depressive symptoms during the pubertal transition than Black females ($\beta = 47.05$, $SD = 1.18$, $p < 0.01$). Using multilevel modeling, it was determined that early-developing White females demonstrate a distinct curvilinear pattern, with increased internalizing problems (withdrawn, somatic complaints, anxious/depressed) during early adolescent years and a higher level of internalizing problems at age 15.

Evidence in some studies point to Black adolescents and young adults experiencing greater levels of stress than White adolescents, which in turn may lead to depressive symptoms (Atkins, Wang, Dupre, Van Den Oord, & Elder, 2009; Boardman & Alexander, 2011; Walsemann et al., 2011). In a study examining stressful life events, race, pubertal status, and body image, early maturing Black adolescent males experienced more depressive symptoms, especially when under higher levels of stress. In the same study, the results of developing depressive symptoms were also true for White adolescent females experiencing early puberty and higher levels of stress. Depressive symptoms in adolescent females in this study were shown to be related to negative body image (Hamlat, Stange, Abramson, & Alloy, 2013). In a meta-analysis, there was a strong relationship between depressive symptoms and racism in Black adult males (Pieterse, Todd, Neville, & Carter, 2012), but it is not known how this experience may differ in Black males as compared to females.

In a study examining racial differences between White and Black adolescents, researchers found that White adolescents were more likely to be fearful at school, particularly in an urbanized city, thus experiencing greater perceived stress. In both races, adolescents who had been previously bullied were more fearful of being bullied again. If a school had rules that were strictly enforced, White adolescents had a decreased
perception of fear while at school. If there was gang presence at school, White adolescents also reported more concerns of their safety in school. One final variable that caused fear of victimization for White adolescents but not Black adolescents was living in a central city (Bachman, Randolph, & Brown, 2011).

Race/ethnicity has also reportedly been related to cortisol (DeSantis, et al., 2007). In the DeSantis and colleagues (2007) study, Blacks had a flatter diurnal cortisol rhythm as compared to Whites. Cortisol differences were also seen in Hispanics, with differences emerging at late adolescence. In Black adolescent males, the slopes of are flatter than in Black females.

In a similar study, researchers found two different categories of Black adolescents related to bullying. The first category consisted of victims of bullying and the second category was called “bully-victims” (an individual who bullies others and is also victimized by bullies). This study included adolescents (n = 10,254) ages 11-15, with 19% primarily urban Black adolescents; one-third of adolescents reported being bullied about the way they talked, looked, or dressed. The next most common reason for being bullied was related to race or skin color (Goldweber et al., 2013). In an urban setting, Black adolescents were more likely to be bullied about money than those who were living in non-urban settings. Among Black adolescents, peers tend to support aggressive behaviors and are less likely to seek help from adults (Shirley & Cornell, 2012).

In a different study, researchers found that Black adolescents and other ethnic minority groups were more likely to bully and less likely to be victims (Wang, Iannotti, & Nansel, 2009). Black adolescents are more likely to be afraid of victimization in non-urban settings than in urban settings (Bachman, 2011). In a Canadian study, Larochette,
Murphy, and Craig, (2010) found that Black-Canadians adolescents were not only involved with bullying, but were also victimized related to how they dressed. Other studies in the United States show that Black children and adolescents are not victimized as often as other ethnic groups (Hanish & Guerra, 2000; Sawyer, Bradshaw, & O'Brennan, 2008; Spriggs, Iannotti, Nansel, & Haynie, 2007).

From a different perspective, researchers examined adolescents ($n = 954$) in four different racial/ethnic groups (Asian, Black, Hispanic, and White) and found that race/ethnic origin was not related to disparities in mental health (Rosenthal & Cody-Wilson, 2012). A concern of Rosenthal and Cody-Wilson (2012) in their review of 24 studies from 2000 to 2010 was that although there may have been statistically significant findings in other studies, these findings may be related to small effect sizes in large samples without controlling for other contexts such as geographical location, community, and historical issues. One additional study by Kubik, Lytle, Birnbaum, Murray, and Perry (2003) found when examining the prevalence and correlates of depressive symptoms in adolescents, Native-American adolescents had the highest percentage of elevated depressive symptoms (CESD-20 score of 16 or greater) at 52%; whereas, Whites had the lowest level of depressive symptoms at 30%. Asians ranked second highest at 47%, then other races at 45%, Blacks at 44%, multi-ethnic at 42%, and Hispanics at 40%.

Socioeconomic status

In a national study examining over 1,000 adolescents, researchers found that adolescents living in lower socioeconomic areas with fewer mental health providers received less assistance for problems such as depressive symptoms (Cummings, 2014). In another study examining depressive symptoms, health, and socioeconomic status in
Korean high school and middle school adolescents \((n = 69,196)\), Jeon, Ha, and Choi (2013) found lower socioeconomic household status predicted poor health, depressive symptoms, and suicidal ideation. Specifically, female adolescents as compared to male adolescents had more depressive symptoms (43% vs. 31.4%) and suicidal ideation (23.1% vs 14.7%). Male adolescents whose parents had graduated from high school were less likely to report depressive symptoms \((OR: 0.81, 95\% CI: 0.76 – 0.86)\) or suicidal ideation \((OR: 0.83, 95\% CI: 0.76 – 0.90)\).

Furthermore, in a study with Chinese-American adolescents, researchers reported that family economic strain predicted depressive symptoms in later adolescence \((\chi^2, 152, n = 444) = 318.9, p < 0.001, CFI = 0.92, RMSEA = 0.050; SRMR = 0.037\) (Mistry, Benner, Tan, & Kim, 2009). In a similar study with 7th grade adolescents \((n = 3,621)\), Kubik and colleagues (2003) found that socioeconomic and minority status were independently related to depressive symptoms. Over 40% of low SES adolescents reported depressive symptoms when compared to 25% of adolescents who were in the category of high SES. In this study, SES was measured using an algorithm developed by the researchers involving school lunch status, parents’ educational level, adolescents’ living arrangement status, and number of parents working full time.

Socioeconomic status (SES) has also been examined in relation to diurnal cortisol levels. In a study by DeSantis and colleagues (2007), SES was examined in relation to cortisol levels and found to have a non-significant relationship \((SD = 0.28, \text{ flatter}, p > 0.10)\). Lupien and colleagues (2001) examined children and adolescents ages 6 to 16 years \((n = 307)\) to determine if there was an association between SES, mental health, and stressful life events. SES was measured by annual salary, education level of parents, and
employment type and time (full time or part time). Results indicated that children ages 6 to 10 from low SES had significantly higher salivary cortisol levels when compared to high SES status children \( F (1,307) = 6.62, p < 0.01 \). There were no SES differences among the high school age adolescents.

**Gender Identity**

The American Psychological Association (2011) defines gender identity as “one’s sense of oneself as male, female, or transgender” (p. 1). Gender identity can influence the variables of stressful life events, perceived stress, cortisol, bullying, and depressive symptoms. In the current study, adolescents were asked about their gender identity and given the options of heterosexual, homosexual, bisexual, or unsure of gender identity. Although the term sexual orientation is the correct term for adolescents in the “sexual minority,” the school principal from one of the high school preferred to use the term “gender identity” when asking students about their sexual preferences.

Collier, Bos, & Sandfort (2013) discusses that an adolescent is considered in the sexual minority, that the adolescent has a same sex attraction; same sex encounters, or identifies themselves as lesbian, gay, bisexual, queer, or unsure of his/her sexual or gender identity. Another term, gender minority is also used to refer to those who are gender non-conforming or are transgender. The abbreviation of LGBT is intended to represent those adolescents who consider themselves lesbian, gay, bisexual, or transgender (Collier et al., 2013).

There has been a research focus since the 1980’s on sexual and gender-minority adolescents (U. S. Institute of Medicine, 2011). During this time, results from studies report that there are psychosocial and health issues related to the stress of victimization
among those adolescents who are homosexual, bisexual, transgender, or unsure of their
gender identity (Hardy, 2007; Martin & Hetrick, 1988; Radkowsky & Siegel, 1997;
Savin-Williams, 1994). Victimization rates as high as 33% have been documented by
researchers (Katz-Wise & Hyde, 2012), and sexual minority adolescents are about 1.7
times more likely to report assault at school (Friedman, Koeske, Silvestre, Korr, & Sites,
2006). Furthermore, rates of suicidality and depressive symptoms are also documented at
higher levels in a recent meta-analysis (Marshal et al., 2011).

Homosexuality in many societies is highly stigmatized. This is also known as
heterosexism or sexual prejudice (Herek & McLemore, 2013; Norton & Herek, 2013).
Evidence indicates that lesbian, gay, bisexual, and transgender (LGBT) adolescents who
live in communities or social environments that are less protective of those in the sexual
minority are more likely to experience depressive symptoms and more likely to attempt
posit that changes in acceptance in LGBT adolescents must begin in the schools. Other
important areas include the community or neighborhood where LGBT adolescents live
(Oswald & Holman, 2013).

LGBT adolescents that are ethnic minorities such as Blacks, Hispanics, or Asians
may face additional stress as they are trying to establish their sexual, cultural, racial, and
ethnic identities (LaSala & Frierson, 2012; Tremble, Schneider, & Appathurai, 1989).
Young LGBT adolescents may develop problems with emotional regulation due to
experiencing stress related to a non-heterosexual orientation (King et al., 2008; Meyer,
2013).
**Pubertal Status**

Puberty is a time associated with many transitions that may include physical, emotional, and psychological changes. The primary purpose of these changes is to ensure the ability for human reproduction. Along with a peak in strength, fitness, and speed, there may also be differences in social and biological maturity (Patton et al., 2007). Sexual maturation or gonadarche begins with a pulsatile nocturnal release of gonadotropin releasing hormone. This activity results in growth and development of secondary sexual characteristics (Delemarre-van de Waal, 2002).

It is widely known that females mature physically earlier than most males, particularly with a growth spurt and secondary sexual characteristics such as breast budding, pubic hair, and starting of menstruation (Greil & Kahl, 2005; Shirtcliff, Dahl, & Pollak, 2009). The pubertal period generally lasts approximately 2-4 years after gonadarche begins (Marshall & Tanner, 1969; Marshall & Tanner, 1970, 1986). During adolescence, puberty, and menarche, cortisol may be higher in the day-time and exhibit a steeper diurnal decline after a female begins menstruation (Adam, 2006).

Along with the physical changes, puberty is also known as a time of heightened emotions with the physiological processes driving the adolescents’ psychological and social development (Dahl & Gunnar, 2009). The response of the HPA axis is more likely to occur when there is an uncontrollable event or a situation that involves emotional distress (Kirschbaum & Hellhammer, 1994). Social attachment to peers becomes a major goal for many adolescents. In addition, parental conflicts may occur related to behavioral risk or sensation-seeking activities (Steinberg, 2005). Researchers recognize that stress during the pubertal period can cause changes in brain development, which may make the
adolescent more vulnerable to psychopathology (Romeo & McEwen, 2006; Spear, 2009). Adolescent self-harming behaviors are known to peak around the age of 15 for females, and there may also be significant increases in depressive symptoms, sexual activity, and substance abuse (Hawton, Saunders, & O'Connor, 2012; Patton et al., 2007; Spear, 2013).

Evidence points to the importance of recognizing depressive symptoms in adolescence as these symptoms generally continue into adulthood and may result in a mental illness (Fergusson, Horwood, Ridder, & Beautrais, 2005a, 2005b; Meadows, Brown, & Elder, 2006). Puberty occurs during a time of significant brain growth or “neural plasticity” (Patton & Viner, 2007). Stress during this crucial time in the form of stressful life events, perceived stress, or bullying can change brain development thereby resulting in emotional sensitivities and possible psychopathology (Burghy et al., 2012; Gunnar & Quevedo, 2007; Rahdar & Galván, 2014).

Limitations

Conducting research by using a survey is a convenient way to collect data, and this method has been used primarily in the above studies. Polit and Beck (2012) posit that surveys rarely delve into complex issues; yet in the above studies, many of the issues are sensitive and could induce stress during the data collection period. Interview information is thought to be the best method of collecting survey data in order to achieve quality information from participants. An advantage of a survey interview is also that participants are less likely to refuse participation (Polit & Beck, 2012). In the current study, conducting one-on-one survey interviews would not be feasible in a school setting due to time limitations. Furthermore, depending on the data that is needed, privacy and
space to meet with adolescents could also be a problem (Stalter, Chaudry, & Polivka, 2010).

Another limitation with collecting data in a school setting or location is when the parent and the adolescent are not physically together when providing consent for participation. Sending paperwork home with adolescents and expecting the adolescents to return the forms can be difficult unless there is something that the adolescents want to gain by being in the study (Unger et al., 2004).

Unger and colleagues (2004) examined 6th grade adolescents (n = 4,427) related to smoking. Two methods of consent were used, one method was active consent, where the adolescent had to take the consent form home and return it to school. The second method was passive consent where the information was sent home with the adolescent, but the parents had to send back a written refusal if they did not want their 6th grader to participate. In this study there were 76% of the participants who returned the signed parental consent, 9% of parents actively refused, and 15% provided implied consent (non-response). Adolescents who returned their signed consent form received a goody bag of small gifts (pens, stickers etc.) and teachers received a $25 gift certificate for helping to remind adolescents to return their forms. Duplicate consent forms were distributed one week later when adolescents did not return their forms with researchers returning weekly to collect forms and redistribute those not turned in. Although the use of active consent was successful in this study, the applied consent increased the participation rate from 76% to 91%. It is likely that in all of the above studies, the consent would need to be active due to the sensitive nature of the questions asked of the adolescents and the fact that almost all studies included minors. The Institutional Review
Board would require active consent for anyone younger than 18 years of age (Duncan, 2009).

The nature of self-report must be considered in the studies discussed above involving the variables of stressful life events, perceived stress, bullying, and depressive symptoms in 9th grade adolescents. On a busy school day when data is collected, there are distractions and pressures to conform to other adolescents that may influence what a 9th grader or other-age adolescent provides as data on a survey or an interview data collection period. In addition, events can occur during the day that could cause cortisol to rise unexpectedly such as a fire drill, school lock down, or tornado warning. If data is collected at a clinic setting, the researcher is dependent on the clinic schedule for appointments to meet with adolescents with no idea of situations that could have occurred prior to coming for the visit. Very few of the above studies mentioned providing incentives to the adolescents. It is important not to provide a large incentive that could unduly influence the adolescent; yet, something of value to the adolescent must be provided so as to peak their interest enough to participate (Polit & Beck, 2012).

Summary

Depressive symptoms in a 9th grade adolescent population has been identified as an issue that should be monitored more closely when this age group transitions from middle school to high school (Cushman, 2006). The literature clearly reports that depressive symptoms can lead to a diagnosis of clinical depression if symptoms are left unchecked and intervention is not provided (National Institute of Mental Health, 2012).
The cost to the United States and to the quality of life of an adolescent is considerably more than it would cost to do a simple screening with follow-up studies if needed to help recognize the problem early in high school (National Research Council and Institute of Medicine, 2009). Many issues can lead to depressive symptoms in a 9th grade adolescent population; some of these issues include stressful life events, perceived stress, bullying, race, gender, socioeconomic status, gender identity/sexual orientation, or pubertal status. Each of these variables and covariates has been discussed in regard to the effect that each one has on depressive symptoms. It is not feasible to screen adolescents for everything that could lead to depressive symptoms, but screening is something that has been recommended by the U.S. Congress and would be a step in the right direction (U. S. Preventative Services Task Force).

Collecting a physiological measure that will quantify the amount or level of stress that the adolescent is experiencing is an innovative approach that has been suggested by leading researchers in the field of biobehavioral medicine (Granger et al., 2012; Juster et al., 2011; McEwen, 2013; Rudolph et al, 2011; Shirtcliff et al., 2012). Although collecting four or more samples of salivary cortisol in a day would be most desired for evaluating the cortisol diurnal rhythm, the cost and feasibility of collecting such large amounts of data in a school setting would not be feasible either economically or physically.

This current study fills a gap in the literature in that the analysis and reporting of the data collected will help individuals who are working with adolescents in the 9th grade to recognize the importance of examining more than one measure of psychological or psychosocial experience. Including a physiological measure such as salivary cortisol
provides a better view of the level of stress the adolescent is experiencing and helps school nurses and psychology professionals to more fully understand what “gets under the skin and becomes embedded” in the future trajectory for this population (Doane et al., 2013 p. 641).
CHAPTER 3
METHODOLOGY

This study examined stressful life events, perceived stress, and bullying to determine the influence of these variables independently and cumulatively on depressive symptoms in 9th grade adolescents. In addition, cortisol (physiological biomarker of stress) was examined to determine if cortisol diurnal rhythm has a mediating relationship with each of the independent variables (stressful life events [SLE, LCU], perceived stress, and bullying), and the dependent variable, depressive symptoms. The confounding variables of gender, race, gender identity, socioeconomic status, and pubertal status were controlled for statistically in this study.

In this chapter, the research design, sample and setting, instrumentation, data collection methods, protection of human subjects, data management, and data analysis are discussed. A pilot study (n = 31) was completed to address feasibility issues of collecting data in a high school setting and to inform the present study. Details of the pilot study are also discussed in this chapter.

Research Design

A descriptive, cross-sectional, correlational design with a biobehavioral focus was used to address the following research questions: (1) What are the descriptive characteristics of the measures of stressful life events, perceived stress, cortisol diurnal rhythm, bullying, and depressive symptoms in 9th grade adolescents; (2) Are there
correlations between stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms in 9th graders; (3) Do stressful life events, perceived stress, bullying, and cortisol diurnal rhythm influence depressive symptoms in 9th graders; (4) Does cortisol diurnal rhythm mediate the relationship between stressful life events and depressive symptoms in 9th graders after controlling for gender, race, socioeconomic status, gender identity, and pubertal status; (5) Does cortisol diurnal rhythm mediate the relationship between perceived stress and depressive symptoms in 9th graders; (6) Does cortisol diurnal rhythm mediate the relationship between bullying and depressive symptoms in 9th graders?

Descriptive studies elucidate the relationships among the variables by examining a sample of the population. In cross-sectional studies, data are collected at one point in time, and in a correlational design, the researcher explores the interrelationships among variables of interest (Polit & Beck, 2012). Data from correlational studies are most appropriate when theory guides the analysis or when there is evidence to indicate that one variable comes before another. Cross-sectional studies are economical and can allow for inferences about events progressing over time; however, a cross-sectional design is generally less persuasive than a longitudinal design. Correlational and cross-sectional design research must be examined with caution; that is, for true causality to be inferred, the researcher must have control over the independent variables (stressful life events, perceived stress, and bullying) (Polit & Beck, 2012).

In this study, these variables cannot be controlled because these events have either already occurred or the events are ongoing. In addition, there are many other variables that could also influence depressive symptoms in a 9th grade population. It would not be
feasible to measure and collect data on all variables that could influence depressive symptoms in a high school setting.

Setting and Sample

Characteristics of the Sample

This study used a non-probability, convenience sampling method, recruiting 9th graders from two public suburban high schools. In school settings, convenience sampling is a common technique allowing for easier access to participants; however, interpretation of results must be viewed cautiously due to possible over and underreporting of sensitive issues (Cornell, Klein, Konold, & Huang, 2012; Tourangeau & Yan, 2007; Unger et al., 2004; Wyrick & Bond, 2011). This age group (14-16 year old 9th graders) is important to study because of the multitude of issues (puberty, peer relationships, bullying, stressful life events) that can occur during adolescent development and the transition to a high school setting (Langdon & Preble, 2008; Wills, Roberts, Backett-Milburn, & Lawton, 2012). In addition, Erikson’s stage of identity vs. role confusion is a crucial period when a 9th grader is trying to determine where he or she fit in or belong in the social environment and learning to develop a sense of self-worth (Anderman & Freeman, 2004; Erikson, 1968).

If problems are occurring with stressful life events, perceived stress, bullying or depressive symptoms, 9th graders generally do not disclose problems unless they are asked specifically (Cushman, 2006). Even then, 9th graders may not be honest or reveal current personal problems due to the sensitive nature of such issues as bullying, stressful
life events, pubertal changes, or depressive symptoms (Duncan, Drew, Hodgson, & Sawyer, 2009; Langhinrichsen-Rohling, Arata, O'Brien, Bowers, & Klibert, 2006).

**Inclusion and Exclusion Criteria**

The following eligibility requirements for this study included: (1) 9th graders aged 14-16 years; (2) capable of signing assent and participating in the study; (3) capable of understanding, speaking, and responding in English, (4) capable of responding to instruments and following instructions for collecting saliva, and (5) having parental consent. Exclusion criteria included: (1) non-English speaking; (2) self-report of pregnancy; (3) unable to complete instruments (4) self-report of clinical depression, Bipolar disorder, Cushing’s disease or Addison’s disease; (5) self-report of taking medications that would affect cortisol levels (e.g., oral contraceptives, oral or inhaled corticosteroids for asthma, etc.), and (6) physical illness with resulting self-report of elevated temperature.

Evidence supports that the above criteria would be appropriate for this study with regard to medication effects on cortisol levels (Hibel, Granger, Kivlighan, & Blair, 2006b), history of depression or Bipolar affecting depressive symptoms outcomes in adolescents (Hammen et al., 2000), and Cushing’s disease, Addison’s disease, pregnancy or elevated temperature affecting cortisol levels (Brown, Rush, & McEwen, 1999; Brown, Varghese, & McEwen, 2004; Chan, Carrell, Zhou & Read, 2013; Karatsoreos et al., 2010).
**Characteristics of the Setting**

The setting of this study was two public high schools located in two small suburban towns in the U. S. Southern Gulf Coast area. The concept of suburban is defined as a residential district located on the outskirts of a city (Webster, 1984). As of 1950 census, the urbanized area concept was adopted to better account for increased growth in suburban areas located outside incorporated areas with a population of 50,000 or more. A suburb is a population that lives in a metropolitan area, outside of a major city. An area would be called suburban or urbanized if there are 1,000 to 3,000 persons per square mile (U. S. Census Bureau, 2013). In the community of School A, the population estimate in 2013 was 13,710. In the community of School B, the population estimate in 2013 was 6,156. The two schools selected were similar in racial and socioeconomic characteristics. School A has approximately 1000 adolescents with 265 enrolled in 9th grade.

Demographic characteristics in school A included 81% White, B8% black, 2% other races. There were 51% of students who free or reduced-cost lunches and 49% who received full-pay lunch (D. Collins, personal communication, June 5, 2014). School B has approximately 450 adolescents with approximately 100 enrolled in 9th grade. Demographic characteristics for school B included 85% White, 12% Black, and 3% other races. There were 44% of students who received free or reduced-cost lunch, and 56% who received full-pay lunch (J. McCutchen, personal communication, June 5, 2014).

**Sample Size and Justification of Sample Size**

The chosen study sample size involved desired power, alpha level, and expected effect sizes. Failure to consider these can lead to inability to find a significant effect
(expected mean difference). A significance level is normally set at $\alpha (p < 0.05)$ as the probability of committing a Type I error and a beta ($\beta$) or probability of committing a Type II error of 0.80. A power analysis is used to reduce the number of type II errors and strengthen the statistical conclusion validity by estimating in advance the number of participants needed (Polit & Beck, 2012).

In addition to the target sample size, the researcher should consider the number of participants who will be recruited to obtain the actual sample size needed. Polit and Beck (2012) suggest that the researcher should attempt to recruit double the amount of participants needed for the study expecting that at least half of those recruited will not be interested or will not meet criteria. In the pilot study that was conducted in the Fall of 2013, two classrooms of 9th graders were recruited with approximately 30 adolescents in each classroom. Out of those asked to participate, approximately 41 took home a packet of information to ask for parental consent. Thirty four 9th graders participated in the study with three being excluded once the study data had been collected with an attrition rate of less than 10%. Recruitment attrition of the pilot study was approximately 52%.

For the current study, a power analysis with a statistical program G-Power (Faul, Erdfelder, Lang, & Buchner, 2007) was completed. For multiple linear regression, the G Power-3 calculation estimates a sample size of 135 with a power of 80% to detect a medium-effect size of 0.30 (See Figure 2). Attrition must be considered as some participants may agree to participate initially but may later change their minds or be unable to participate for different reasons. This can be a problem because participants who drop out may differ in significant ways from those who stay in the study (Polit & Beck, 2012). Since data collection for this study occurred all in one day at a school
setting, the attrition rates were expected to be minimal as seen in the previous pilot study (< 10%). Based on the above calculations, a sample size of 144 was enrolled in the study to allow for attrition (10%), missing data, or inadequacy in saliva collection for participants.

Figure 3. Power Analysis: Multiple linear regression

Note. Fixed model, $R^2$ deviation from zero. A priori input effect size $f^2 = 0.149$, alpha=0.05 and with 80% power with fourteen predictors. Calculations were conducted with G-Power-3 software (Faul, Erdfelder, Lang, & Buchner, 2007).

Protection of Human Subjects

Informed consent is required for the safety of research participants of any age but particularly for children and adolescents due to their vulnerable status (Code of Federal Regulations, 2009; Chartier et al., 2008; Fisher, 2004). Ninth graders are considered a vulnerable group because they are minors and could be subject to coercion or enticement to participate in a research study (Kopelman, 2004). Previous studies have reported that 9th graders are cognitively able of providing their own assent in the research process (Kopelman, 2004; Lind, Anderson, & Oberle, 2003). The researcher must have knowledge of cognitive ability and development in order to understand a 9th grader’s
motivation to participate (Iltis, Matsuo, & DeVader, 2008; Wendler, 2006). Ninth graders have a unique perspective as they are living the experience of transitioning to a new school, may be more vulnerable to bullying, may experience stress that comes along with making new friends, may experience more difficult academics, and are likely to be undergoing physical and hormonal changes (Cushman, 2006; Thornberg & Knutsen, 2011; Tram & Cole, 2000).

The pilot study was approved by the Institutional Review Board (IRB) at the University of Alabama at Birmingham (UAB) in the Fall of 2013 including all protocols, informed consent, study measures, parental information, and reminder letters to 9th graders for the pilot study (see Appendix C). The school system utilized in the pilot study also approved all documents. The current study was reviewed and approved by the UAB IRB on August 25, 2014 as well as approved by the school systems, including the superintendent, counselors, and school nurses.

For the current study, an amendment to the pilot study was submitted to the IRB including the following changes: (1) Information packets were hand-carried to the parents by the 9th graders and hand-carried back to the school office and placed in the principal investigator’s locked box; (2) Screening forms were completed at the school by the student in a private setting once the consent form was signed by the parent; (3) Ninth graders were given a small incentive to encourage the return of the signed packets (pencil, pen, and highlighter – less than $1.00 per person) regardless of meeting the inclusion/exclusion criteria once the consent form was returned and the screening form was complete. The consent form was signed by both the 9th grade participant and at least one parent. If a 9th grader declined participation after a parent provided consent, he or she
was thanked for their interest and time, and the investigator documented the 9th grader’s decision to decline to participate.

The reasoning behind the amendment to the protocol was due to problems in the pilot study getting the consent form and screening form back to the principal investigator. The original protocol for the pilot study called for the consent and screening form to be mailed back to the principal investigator due to the confidential nature of the screening form. The online questionnaire for the pilot study also included the screening questions for inclusion/exclusion to assess whether the 9th graders were able to accurately complete the screening form. After reviewing the data from the pilot study, the 9th graders were able to provide answers to questions such as “Do you take any daily medications and what are the names of these medications? Are you pregnant? Has the doctor ever told you that you have Clinical depression, bipolar disease, Addison’s, or Cushing’s disease? For the current study these screening questions were given to the 9th graders in the presence of the principal investigator so that if the 9th grader had questions about the screening instrument, the questions could be answered in a private setting. Adolescents that did not qualify for the study were told at that time and provided the small incentive (pen, pencil, and highlighter) for their time and effort.

The principal investigator used the UAB-recommended informed consent document format that has a Flesch-Kincaid Grade level of 11.2 (Paasche-Orlow, Taylor, & Brancati, 2003; Walsh & Volsko, 2008). With the population of 9th graders being age 14-16, the IRB advises that the informed consent document be signed by their parent or guardian and that the ninth grader sign on the same form as an assent to participate.
(Tigges, 2003) (see Appendix F). If either a parent and or the 9th grader did not sign the consent, the 9th grader did not participate.

The study procedures took place in a designated area (school computer lab) to ensure privacy. During completion of the online survey questions, 9th graders were seated in front of individual computers. The computer setting was designed so the 9th graders could not see the screen of others; therefore, this provided a degree of privacy while completing study measures. Ninth graders were allowed to raise their hand for questions during the data collection period and were assisted by the principal investigator or a research assistant.

During the pilot study’s computer data collection period, research assistants and the principal investigator were present to answer any questions that arose related to the online survey. For example, several 9th graders asked what heterosexual meant and also asked what was meant by “body hair growth” on the pubertal status questionnaire. Research assistants were trained in advance as to how to answer these questions, and answers were uniform.

The school counselor and school nurse were informed about data collection procedures and were requested to assist if a 9th grader became upset as a result of responding to any of the survey items. Confidentiality was maintained by the use of a numerical coding system in the database. The names of 9th graders were known only to the principal investigator and the research assistant. Neither the 9th grader’s name nor any identifying information appeared on any of the survey data answered online. However, 9th graders were made aware that anyone who appeared to be at risk of harm to themselves or others after the investigator examined the depressive symptoms
questionnaire would be referred to the school nurse and counselor for immediate follow-up. During the pilot study, the school nurse was present for the period of time that 9th graders completed the online survey. None of the 9th graders showed any signs of being upset before, during, or after completing the online survey.

Pilot Study

A pilot study was conducted during Fall of 2013 to determine feasibility of data collection procedures in the high school environment. Additionally, the pilot study helped to address potential issues with participant recruitment and to inform the current study. This pilot study was funded by Sigma Theta Tau International – Zeta Gamma Chapter.

Sample and Recruitment

The convenience sample was composed of 9th graders (n = 31), aged 14-16 years, who were recruited from a suburban high school in the South. The principal investigator presented the study to the 9th graders in two separate classrooms during an elective class. During the recruitment presentation to the 9th graders, the principal investigator briefly discussed the study goals, data collection measures, and saliva collection for cortisol analysis, as well as the risks, benefits, and incentive for participation ($5.00 gift card to local food establishment). The principal investigator stressed confidentiality throughout the study and provided an opportunity for 9th graders to ask questions.

Consent and Assent

A total of 60 packets (parent letter, informed consent document and screening questionnaire) were distributed. Ninth graders were asked to give the information packets to their parents to obtain parental consent and return the consent form via mail (self-
addressed stamped envelope) to the Principal investigator within one week. Out of the 60 packets that were sent home, 34 (56%) of the parental consent forms were returned, and assent was obtained from 34 (56%) of the 9th graders. The principal investigator met with each 9\textsuperscript{th} grader individually the day before data collection took place to make sure each one understood the pilot study, had an opportunity to ask questions, and signed the assent form. For those who completed the assent process, the principal investigator sent home a letter with the 9\textsuperscript{th} graders reminding them not to eat or drink anything but water one hour before classes began on the day of data collection.

\textit{Data Collection}

The morning of data collection day, the 9\textsuperscript{th} graders from the first-period classroom participating in the study reported to the school computer laboratory. Each 9\textsuperscript{th} grader was given a small cup of water to swish and swallow as to clear their mouth of any debris and was then directed to a personal computer until all participants arrived. While waiting for others to collect saliva, the 9\textsuperscript{th} graders were allowed to use the computer to work on homework.

Groups of six 9\textsuperscript{th} graders were taken into a smaller room to collect the morning saliva sample. The research assistants first discussed the saliva collection procedure “passive drool” and then demonstrated the saliva collection procedure before asking the 9\textsuperscript{th} graders to begin their saliva collection. A timer was set for 3 minutes, and each one was logged in by a research assistant to document the time of starting and time of stopping the saliva collection. One 9\textsuperscript{th} grader needed more than three minutes to collect saliva, so more time was allowed, and this was documented in a log. Once all 9\textsuperscript{th} graders
had collected saliva (at least one ml in a cryo tube), they were escorted back to their seats in the computer lab.

The entire group of 9th graders was then given the link to the Survey Gizmo website for the cumulative survey that had been previously uploaded by the principal investigator. Time of starting the survey and stopping the survey was logged for each 9th grader, and any problems encountered with the link were documented. Several 9th graders had to stop and start the survey over due to a problem with getting “booted” off of the website by the school system. This issue was documented, and once it was resolved by the principal investigator, the 9th graders completed the questionnaire.

Upon completion of the online surveys, 9th graders then came forward one at a time to be signed out and to receive a bottle of water and a choice of a snack (chips, crackers, nuts, etc.) before returning to their respective classrooms. The same procedure for saliva collection was done in the afternoon. Once saliva collection was completed, the 9th graders were provided a $5.00 gift card to a local food establishment as a thank you for participating as well as an information sheet about counseling services available should any 9th grader need support.

The morning data collection period was completed within one 50-minute classroom period, and the afternoon data collection period was completed within 10 to 15 minutes. Researchers collected data with twenty-four 9th graders on the first day of data collection scheduled. The second data collection day included ten more 9th graders. This second data collection took place approximately two weeks after the first data collection day.
Results

Thirty-four 9th graders or 56% of the 9th graders who took home a packet of information about the study turned in signed consent forms and provided assent. Three 9th graders were excluded due to failure to meet the inclusion/exclusion criteria. The final sample consisted of 31, 9th graders; 16 were male and 15 were female. The mean age for males was 14.4 years and 14.5 years for females with a range from 14 to 16 and SD of 0.67 years. In number of participants in the three age groups were as follows: age 14 (n = 21, 67%), 15 (n = 7, 23%) and 16 (n = 3, 10%). The number of participants in each ethnic group was as follows: White (n = 22, 71%); Black (n = 5, 16.1%); other (n = 4, 12.9%). Gender identity or sexual orientation was self-reported by adolescents as the following: heterosexual (n = 26 - 83.9%); bi-sexual (n = 3, 9.6%) and unsure (n = 2, 6.5%).

The total enrollment for 2013-2014 school year was approximately 265 adolescents for 9th grade with 42% of 9th graders participating in the National School Lunch Program receiving either free or reduced-cost lunch (Harwell & LeBeau, 2010). In the pilot study sample, the socioeconomic status/National School Lunch Program results were as follows: free lunch (n = 12, 38.7%), reduced lunch (n = 1, 3.2%), bring lunch from home (n = 3, 9.7%), full pay for lunch (n = 15, 48.4%). The categories for race, gender identity/sexual orientation and SES were collapsed into two groups for using t-test analysis.

Pubertal status was reported using the Self-Administered Rating Scale for Pubertal Development (Carskadon & Acebo, 1993). An example question (Likert Scale) is the following: Would you say your growth in height ...a) has not yet begun to spurt = 1, b) has barely started = 2, c) is definitely underway = 3, d) seems completed = 4, e) I
don’t know = 0. Each item is scored from 1= no development to 4= development completed. The fifth answer of “I don’t know” is considered missing data or “0”. If adolescent females marked a “yes” on menstruation, this answer was given a score of 4. If “no” was marked on menstruation, this answer was given a score of “1”. Male participant self-report scores were as mid-pubertal to late pubertal status ($\bar{x} = 8.0$, $SD=1.32$, Range 6 to 10). Female participants pubertal status self-report scores were at late pubertal to post-pubertal status ($\bar{x} = 11.33$, $SD, 0.976$, Range 10-13). Other data collection measures in the pilot study included the following: The Center for Epidemiologic Studies Depression Survey-10 (CESD-10) (Sakakibara, Miller, Orenczuk, & Wolfe, 2009), The Perceived Stress Scale - 4 (PSS-4) (Cohen, & Williamsson, 1988), and the Personal Experiences Checklist (PECK) (Hunt et al., 2012).

The CESD-10 is a 10 item questionnaire with responses ranging from 0 to 3 and a maximum score of 30 ($\alpha = .87$). The CESD-10 self-report scores were ($\bar{x} = 9.8$, $SD = 6.44$, range, 0 to 23). The PSS-4 survey consisted of 4 questions that had a range of responses from 0 to 4 with a total maximum score of 16 ($\alpha = 0.64$). Self-report scores from the PSS-4 were ($\bar{x} = 6.8$, $SD = 2.8$, range, 0 to 12). The PECK survey consisted of 32 questions on four types of bullying with a maximum score of 128 ($\alpha = 0.90$). Self-report scores from the PECK were ($\bar{x} = 12.9$, $SD = 11.36$, range, 0 to 48) for the pilot study. Subscale pilot study data resulted in the following: Verbal/relational bullying subscale consisted of 11 items ($\alpha = 0.88$, $\bar{x} = 8.1$, $SD = 6.98$). Cyberbullying/electronic subscale consisted of 8 items ($\alpha = 0.69$, $\bar{x} = 1.09$, $SD = 1.66$). Physical bullying subscale consisted of 9 items ($\alpha = .70$, $\bar{x} = 2.2$, $SD = 3.03$). Cultural bullying subscale consisted of 4 items ($\alpha = 0.63$, $\bar{x} = 1.35$, $SD = 2.4$).
General linear models were used to analyze the covariates for Model 1. The independent variables were added in Model 2, and Model 3 contained the cortisol diurnal rhythm results to determine how much of a variance was accounted for with relationship to depressive symptoms in the three separate models. A fourth model was run with all variables to determine how much the $R^2$ changed from the previous models. Model 1 with the covariates result was significant ($R^2 = 0.391$, Adj $R^2 = 0.270$, $p = 0.022$, $\text{Partial Eta}^2 = 0.391$); however, only gender ($F = 5.09$, df, 1, $p = 0.033$, $\text{Partial Eta}^2 = 0.169$), SES ($F = 4.35$, df = 1, $p = 0.047$, $\text{Partial Eta}^2 = 0.148$) and gender identity ($F = 6.77$ df, 1, $p = 0.015$, $\text{Partial Eta}^2 = 0.210$) were significant out of all the covariates. Model 2 was run with only the independent variables (PSS-4 and PECK) and was also significant ($R^2 = 0.593$, Adj $R^2 = 0.563$, $p < 0.001$). The only variable that significantly contributed to depressive symptoms was the PSS-4 ($F (31.54)$, df, 1, $p < 0.001$, $\text{Partial Eta}^2 = 0.539$).

Model 3 was run on the cortisol diurnal rhythm and was not significant. Model 4 was run with the independent variables (PSS-4 & PECK), covariates (gender, race, gender id, SES, and pubertal status), and cortisol (cortisol diurnal rhythm), and was found to be significant ($R^2 = 0.773$, Adj $R^2 = 0.687$, df = 8, $p < 0.001$, $\text{Partial Eta}^2 = 0.773$). The PSS-4 ($F = 29.379$, df, 1, $p < 0.001$, $\text{Partial Eta}^2 = 0.583$), gender ($F = 8.07$, df = 1, $p = 0.010$, $\text{Partial Eta}^2 = 0.278$), gender id ($F (7.17)$, df = 1, $p = 0.014$, $\text{Partial Eta}^2 = 0.254$), and pubertal status ($F = 6.39$ df = 1, $p = 0.020$, $\text{Partial Eta}^2 = 0.233$) were the only variables and covariates contributing significantly to the variance in depressive symptoms. These results must be considered with caution as the sample was small ($n = 31$).

T-tests were run with gender, race, SES, gender identity, and pubertal status. There was significant gender differences in total CESD-10 scores ($t (29) = -2.94$, df = 29
There were also significant pubertal status differences in CESD-10 scores ($t(29) = -2.40, df = 29, p = 0.023$) with late to post pubertal status having higher CESD-10 scores ($n = 15, \bar{x} = 12.47, SD = 6.08$) than mid-to late pubertal status ($n = 16, \bar{x} = 7.3125, SD = 5.90$). There were no significant racial differences in CESD-10 scores when comparing White to Black/other race categories. There were no significant SES differences in CESD-10 scores. There were no significant gender identity differences in CESD-10 scores. Ninth graders with higher PSS-4 scores had higher CESD-10 scores when CESD-10 was put into two categories of clinically depressive symptoms (yes or no) ($\chi^2 = 25.28, p = 0.008, r_s = 0.684$). Adolescents with higher PECK scores were associated with higher CESD-10 scores although non-significant ($t(28) = -1.623, p > 0.05$). CESD-10 was significantly correlated with PSS-4 ($r = 0.77, p < 0.001$) and Gender ($r = 0.401, p = 0.025$). There were no significant correlations between CESD-10 and race, SES, gender ID, pubertal status, and PECK scores.

Saliva was collected in the morning and in the afternoon to account for the cortisol diurnal rhythm. Cortisol is normally at its highest peak approximately 30 minutes after awakening and slowly declines throughout the day, reaching the lowest point around midnight with a slow steady rise (Gunnar et al., 2009). Salimetrics, LLC analyzed the salivary cortisol samples with a high sensitivity salivary cortisol Elisa immunoassay (EIA) kit.

Morning and afternoon cortisol as well as the difference between morning and afternoon samples (cortisol diurnal rhythm) were positively skewed and required log
transformation. The mean salivary cortisol for morning sample was 0.155µg/dL (range of 0.049µg/dL to 0.438 µg/dL, SD = 0.608) and the mean afternoon sample was 0.093µg/dL (range of 0.022 µg/dL to 0.176 µg/dL, SD = 0.543). A mean cortisol change (AM, PM) was -0.06 µg/dL (range of -0.29µg/dL to 0.07µg/dL, SD = 0.609). Reference ranges included 7am to 9am at 0.021µg/dL to 0.883µg/dL and 3pm to 5pm 0.030µg/dL to 0.259 µg/dL (Salimetrics LLC, 2013). Four participants (12.9%) had a blunted cortisol change or dysregulated diurnal rhythm (less than .01 µg/dL change from morning to afternoon) and 7 participants (22.36%) had opposite diurnal rhythm or dysregulated diurnal rhythm cortisol (morning low and afternoon high). Twenty participants (64.5%) had cortisol that followed a normal diurnal rhythm (a negative change). When cortisol AM, cortisol PM, and cortisol diurnal rhythm was added to the correlation matrix, there were only correlations among the cortisol diurnal rhythm and the AM cortisol ($r = -0.822$, $p < 0.001$) and AM cortisol with PM cortisol ($r = 0.392$, $p = 0.029$).

Conclusion

Data collection procedures (salivary cortisol collection in a school setting, and survey data collection on school computers) were feasible in a school setting. There was one missing data point on the PECK survey (for only one participant); the score was replaced by the average of other scores in this category. Bullying scores overall were low, but relational bullying had higher scores than physical, cultural or cyberbullying. Perceived stress scores varied across participants, but those 9th graders who had higher PSS-4 scores also had higher depressive symptoms scores. Ninth graders who self-reported bullying had higher CESD-10 scores than those who did not self-report bullying.
For ninth graders with CESD-10 scores greater than 9, referrals were made to the school nurse.

*Lessons learned from the Pilot Study*

An additional quantitative measure of stress was suggested after discussion with the dissertation committee. This additional measure was based on the high perceived stress scores and the fact that there may be many other sources of stress; therefore, a stressful life events scale (CLES-A) was included in the final study (Coddington, 1972). In addition, the PSS-4 survey was changed to the PSS-10 survey because of the higher-than-expected perceived stress scores, and the PSS-10 has better reliability and validity than the PSS-4. The screening form from the pilot study had previously been completed at home by the parents and mailed to the principal investigator. The investigator had concerns that the screening form might be lost causing a potential confidentiality breach of sensitive information. Therefore, the process of screening was changed. In the pilot study, the computer survey administered to the 9th graders also included the screening questions to determine if the 9th graders were capable of accurately providing the information such as knowledge of medical diagnosis or medications taken daily. Ninth graders for the current study completed the screening form prior to the date of data collection in a private room with the investigator, allowing for questions, explanation of the study, and the opportunity to decline participation if so desired.

Some of the 9th graders in the pilot study had questions about the word heterosexual; an additional word was included next to this word “straight” with the words “gay or lesbian” included beside the word homosexual. Another question that came up during the computer survey was “what do you mean by body hair?” Therefore, for
clarification, the words “chest, underarm, and body hair” were included on the male version of the pubertal status questionnaire and the words “pubic and underarm hair” were included on the female version of the pubertal status questionnaire. One other demographic question was included asking the 9th graders about their living arrangements. They were asked whether they were living with mom, dad, both parents, grandparents or had other living arrangements, due to evidence that living arrangement may cause increased stress on the 9th grader socially, emotionally, and economically (Heifetz, Connolly, Pepler, & Craig, 2010).

Instrumentation

Data were collected related to the variables in this research study including: stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms. Information regarding inclusion and exclusion criteria was gathered from adolescents on a screening survey prior to enrollment in the current study. Instruments for the current study were selected based on the conceptual framework, reliability and validity, and feasibility of instrumentation (See Appendix A for all surveys).

This study used a biobehavioral approach; that is, the investigator explored links among biological, psychosocial, and behavioral variables as they related to mental health outcomes (Kang, 2003). The National Institute of Nursing Research and the National Institutes of Health support this method in their strategic plan for the upcoming years as this research approach allows us to see how patterns of behavior in different populations may begin with an individual’s biology, genetic make-up, and environmental influences (Grady, 2006). Study variables and corresponding measures are summarized in Table 1.
Table 1

Study Variables and Corresponding Measures

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<tr>
<th>Variable</th>
<th>Variable Type</th>
<th>Measure</th>
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<tr>
<td>Depressive symptoms</td>
<td>Dependent</td>
<td>Center for Epidemiological Studies Depression Survey-10</td>
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<td></td>
<td></td>
<td>(CESD-10)</td>
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<tr>
<td>Stressful life events</td>
<td>Independent</td>
<td>Coddington Life Event Scale – Adolescents (CLES-A)</td>
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<td>Perceived stress</td>
<td>Independent</td>
<td>Perceived Stress Scale-10 (PSS-10)</td>
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<td>Bullying</td>
<td>Independent</td>
<td>Personal Experience Checklist (PECK)</td>
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<td>Covariate</td>
<td>Demographic Questionnaire</td>
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<tr>
<td>Race</td>
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<td>Covariate</td>
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<td>(National School Lunch</td>
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<tr>
<td>Pubertal Status</td>
<td>Covariate</td>
<td>Pubertal Self Rating Scale</td>
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</table>

Demographic Questionnaire

The investigator developed a demographic questionnaire based on a review of literature to identify key demographic variables. This questionnaire, given at the beginning of the online survey, included the following questions: what is your age (options: 14, 15, 16); what is your gender (options: male or female); what is your race? (Options: White, Black, other); what is your socioeconomic status (National School Lunch Status) (Options: free or reduced-cost lunches, full pay or bring lunch from home); what is your gender identity (Options: heterosexual or straight, homosexual or gay, bisexual, or unsure). Adolescents were also asked questions at the beginning of the survey.
as these items could provide evidence as to unusual patterns in the cortisol levels. The questions were: Are you a smoker (options are “yes” and “no”) with a follow up question: what day and time did you last smoke?, What time did you wake up?, and Have you had anything to eat or drink besides water in the last hour?, Do you have a fever or have you been sick and out of school in the last few days?, Are you pregnant?, Are you on any daily medications?, Have you exercised today? ; If you have exercised, what type of exercise did you do? And what are your living arrangements? (Options: live with both parents, live with one parent, live with grandparents, other living arrangement).

An additional question (Has anything stressful occurred to you today? [Stressful Day]) was added at the end of the second data collection period to help in understanding the levels of morning and afternoon cortisol and to further substantiate the 9th grade transition experience.

The screening questionnaire, developed by the investigator, based on the literature review, was used to screen potential participants for the inclusion/exclusion criteria. These questions were asked of the 9th graders in a private setting once the parent had given consent for the 9th grader to participate in the study. Questions on the screening questionnaire included: What is your full name; What is your current age; Do you take any medications daily; Have you even been told that you have a diagnosis of Clinical Depression, Bipolar disorder, Addison’s disease, or Cushing’s disease; Are you pregnant (Females only); Are you a smoker?, and Have you had an illness in the last few days that caused you to have fever where you had to stay home from school?; Once a 9th grader completed the screening questionnaire with the principal investigator, he or she was
given a small thank-you gift (less than $1.00 per person – Pen, pencil, highlighter) regardless of whether the 9th grader was eligible to participate in the study.

The Center for Epidemiological Studies Depression Survey-10

Depressive symptoms were measured with The Center for Epidemiologic Studies Depression Survey (CESD-10), which has been used to measure four factors related to depressive symptoms including: positive/negative affect, somatic symptoms, retarded activity, and interpersonal issues as defined by the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM-IV) (Al Kalaldeh & Abu Shosha, 2012; Hales, Dishman, Motl, Addy, Pfeiffer, & Pate 2006). The CESD-10 was originally developed in 1993 as a shorter version of the 20-question CESD (Kohout, Berkman, Evans, & Cornoni-Huntley, 1993). This 10-item Likert scale ranges from 0 (rarely) to 3 (all the time), yielding a total score of 0 to 30, with a score of 10 or greater indicating need for referral (clinically meaningful) (Raffaeli et al., 2013)

The CESD-10 is widely used in psychiatric epidemiology in screening for depressive symptoms in all ages. This measure is publically available. Other measures of adolescent depressive symptoms include the Children’s Depression Inventory (Kovacs, 2004), the Beck Depression Inventory (Beck, Steer, & Brown, 1996), and the Reynolds Depression Screening Inventory (Reynolds & Kobak, 1998). The CESD-10 was chosen due to the applicability for adolescent populations, the length of the measure being only 10 questions as opposed to the longer length of other measures of depressive symptoms, and the acceptance of this measure by the American Psychiatric Association Diagnostic and Statistical Manual (DSM-V).
The CESD-10 showed accuracy in classifying individuals with depressive symptoms in a sample of $n = 563$ ($Kappa$ $-0.082$, $p < 0.001$). Sensitivity was 91% and specificity was 92% with a positive predictive value of 92% (Zhang et al., 2012). When comparing the CESD-10 to the CESD-20, the internal consistency was $\alpha = 0.91$ for CESD-20 and $\alpha = 0.86$ for CESD-10. The CESD-10 was again compared to the CESD-20 and showed an internal consistency reliability coefficient ($\alpha = 0.88$). The CESD-10 has a reliability coefficient using a test-retest correlation ($r = 0.85$) (Bradley, Bagnell, & Brannen, 2010; Bradley, McGrath, Brannen, & Bagnell, 2010). The CESD-10 has been used to assess adolescents’ aged 15-21 ($\alpha = 0.87$) in the depressive affect and somatic retardation questions. In another confirmatory factor analysis study, internal consistency was $\alpha = 0.75$ in a study of 269 adolescents (mean age of 13.9) (Cartierre, Coulon, & Demerval, 2011). Internal consistency of the CESD-10 for the pilot study was ($\alpha = 0.87$).

**Coddington Life Event Stress Scale for Adolescents (CLES-A)**

Stressful life events (SLE) and the impact of these events (LCU) were measured with The Coddington Life Events Scale, which was patterned after the Social Readjustment Rating Scale (SRRS), designed to measure stressful life events. This instrument was originally designed for adults ages 18 and above (Holmes & Rahe, 1967), though revised by Coddington (1972) for several age groups including preschool, elementary, middle, and high school (Coddington, 1972). Coddington (1972) gathered information on “life events” affecting social adjustment in children and adolescents by interviewing teachers, pediatricians, and mental health workers. After examining the scale further, Coddington (1999) readjusted the scale to fit three categories (children, adolescents, and parents). The adolescent version was renamed the Coddington Life
Event Scale-Adolescent (CLES-A) (Coddington, 1972). The CLES-A was produced at a 4th grade reading level using the Dale-Chall procedure (Coddington, 1999). The CLES-A can be completed in approximately 15 minutes (Coddington, 1999). Validation and reliability have been determined by the use of the CLES-A in multiple studies with test-retest reliability (α = 0.84) (Thompson & Morris, 1994) and interclass correlation (r = 0.63) (Villalonga-Olives et al., 2008). Other selected studies using the CLES-A documented α = 0.53 (Coddington, 1980) and α = 0.78 (Wu et al., 2011).

CLES-A was designed for adolescents aged 13-19 years. Only 9th grade adolescents participated in this current study (ages 14-16). Respondents were asked to rate the number of times a stressful life event (SLE) occurred in the past 12 months. The CLES-A takes into account that the effect of a stressful life event may diminish over time; therefore, as participants mark the events occurring on the CLES-A, they must also mark whether the event occurred in the last 3 months (fully weighted), 6 months (3/4 weighted), 9 months (1/2 weighted) and 12 months (1/4 weighted). Higher scores are attributed to the most recent events. There are 50 individual stressful life events on the CLES-A with scores (Life Change Units – LCU’s) ranging from 26 to 101 per event. The LCU score indicates the amount of social readjustment that is required by an adolescent.

The actual number of stressful life events (SLE) was calculated as well as the score for the LCU. There were 21 positive life events and 29 negative events listed on the CLES–A. Some of the positive events could have also been considered negative events depending on the circumstances. Examples would be “Moving to a new school district” or “Getting a summer job.” This ambiguity makes it difficult to truly separate the negative and positive events. The higher-weighted events are considered the more
stressful events and lower weights are considered positive or less stressful events (Terzian, Moore, & Nguyen, 2010). The Coddington Life Event Scale (CLES-A) is a ratio scale completed by self-report or assisted self-report. In the assisted self-report situations, the researcher read the event aloud to the study participant (Coddington, 1972), and the participant answered individually (this was only required for one participant).

**Perceived Stress Scale (PSS-10)**

Cohen’s Perceived Stress Scale (PSS-10), a 10-item Likert scale instrument, measures the degree to which one appraises events as stressful during the last month (Cohen et al., 1983, 2003). The PSS-10 scores range 0 to 40 and scoring is from 0 (never) to 4 (very often). The PSS-10 scores on each question are added together with higher scores indicating higher levels of perceived stress (Cohen et al., 1983). There are several versions of the Perceived Stress Scale, the PSS-14, the PSS-10, and the PSS-4. The PSS-4 was used in the previously described pilot study, though had low reliability ($\alpha = 0.64$). The PSS-10 was chosen for the current study due to the high number of 9th graders in the pilot study who self-reported perceived stress and the need for increased validity and reliability for use in adolescents (Austin, Smith, & Patterson, 2009; Martinelli et al., 2011; Siqueira et al., 2000; Siqueira et al., 2001).

Reliability for the PSS-10 was previously established ($\alpha = 0.85$) (Al Kalaldeh & Abu Shosha, 2012). When compared to the College Students Life Event Scale, there was a correlation of 0.65. With three different groups of college students studied, reliability scores ($\alpha = 0.84, 0.85,$ and 0.86), and test-retest correlation ($\alpha = 0.85$) (Cohen et al., 1983). Other measures of perceived stress include: The Sources of Stress Scale (Soares &
Soares, 2001), the Derogatis Stress Profile (Derogatis, 1984), and the Personal Stress Inventory Assessment (Kindler, 1981). These measures are not routinely used with adolescents. The PSS-10 is an established measure that is used routinely in with adolescents.

*The Personal Experiences Checklist (PECK)*

The Personal Experiences Checklist (PECK) is a 32-item Likert scale instrument measuring physical, verbal/relational, cyber, and cultural bullying. This instrument was developed by Hunt and colleagues (2012) based on the operational features of bullying and examined a wide range of bullying behaviors, including verbal/relational, physical, cultural, and cyberbullying. This self-report instrument is scored on a range of 0 to 4: 0 (never), 1 (rarely), 2 (sometimes), and 3 (most days), and 4 (every day). Eleven items assess verbal-relational bullying, eight items assess cyberbullying, nine items assess physical bullying, and four items assess cultural bullying. High scores indicate more bullying (Hunt et al., 2012).

This measure was validated with the Olweus Bully/Victim Questionnaire-Revised (OBQV-R), an established bullying measure considered to be the “gold standard” (Olweus, 1994). This questionnaire was studied in adolescents (8-15 years old) with an internal consistency for the four different subscales (α = 0.91) and maximum score of 44 for verbal-relational bullying (α = .91); and maximum score of 48 for cyberbullying (α = 0.90); and maximum score of 36 for physical bullying (α = 0.91); and a maximum score of 24 for bullying based on culture (α = 0.78). Test-retest coefficients were relational-verbal (r = 0.75); for cyber bullying (r = 0.86); for physical bullying (r = 0.61); for cultural (r = 0.77); and for the total PECK scale (r = 0.79) (Hunt et al., 2012).
This scale was chosen because there were no other scales available that measured four types of bullying in one scale. The PECK measure was used in the previously described pilot study ($\alpha = 0.91$). Subscales included the following results for the pilot study: verbal ($\alpha = 0.88$), cyber/electronic ($\alpha = 0.69$), physical ($\alpha = 0.79$), and cultural ($\alpha = 0.63$). Hunt et al. (2012) indicated that PECK appears to be the best scale for this study as it is relatively brief (32 questions) and allows for only one scale to collect data on four documented areas common in the bullying literature as compared to multiple other lengthy and detailed bullying measures in the literature (CDC Compendium of Bullying Measures, 2011).

*Self-Rating Scale for Pubertal Status*

Pubertal status was measured with the Self-Rating Scale for Pubertal Development (PDS) which is a 5-item instrument with 5 ordinal level Likert-type scale responses (Carskadon & Acebo, 1993). An example question is the following: Would you say your growth in height … a) has not yet begun to spurt b) has barely started, c) is definitely underway, d) seems completed, e) I don’t know. Each item is scored from 1 = no development to 4 = development completed. The fifth answer of “I don’t know” is considered missing data or “0.” Carskadon and Acebo (1993) developed this scale as an adaptation of the Pubertal Rating Scale, which involved a verbal interview (Petersen, Crockett, Richards, & Boxer, 1988). This self-report scale provides an estimate of pubertal stage specific to gender. Males are asked two specific questions about deepening of the voice and hair growth on the face with the same Likert-type scale responses. Females are asked three specific questions about breast development, starting menstruation, and age of menstruation.
The instrument classifies adolescents into five categories including pre, early, mid, late, or post pubertal status (Crockett & Petersen, 1987). Total pubertal status scores are determined for males by adding the scores for three questions: body hair growth, voice change, and facial hair growth. The scores for male adolescents are categorized as follows: (1) pre-pubertal = 3, (2) early pubertal = 4 or 5 with no 3-point responses, (3) mid-pubertal = 6-8 with no 4-point responses, (4) late pubertal = 9-11 and (5) post-pubertal = 12. Total pubertal status scores are determined for females by adding the scores for the following three questions: body hair growth, breast development, and menarche. The female puberty scores are categorized as follows: (1) pre-pubertal = 2 and no menarche (score of 1), (2) early pubertal = 3 and no menarche (score of 1), (3) mid-pubertal = >3 and no menarche (score of 1), (4) late pubertal = ≤ 7 and menarche (score of 4), and (5) post-pubertal= 8 and menarche (score of 4).

Tanner (1962) developed the Sexual Maturation Scale, which was designed to be used by health providers to assess pubertal development during a physical examination. The Sexual Maturation Scale today remains the Gold Standard of pubertal rating scales (Schmitz et al., 2004). Validity has been examined for the Pubertal Self Rating Scale, and investigators found that adolescents were consistent in reporting pubertal changes (Petersen et al., 1988). Spearman coefficient shows a strong correlation ($r = 0.868-0.841$) when this scale was compared to other pubertal measures completed by a pediatrician (Carskadon & Acebo, 1993). The overall interclass correlation between the physician ratings and the PDS was $r = 0.61-0.71$ (Brooks-Gunn, Warren, Rosso, Gargiulo, 1987). The correlations between the Sexual Maturation Scale and the PDS were ($r = 0.72$ to $0.80$). The correlations between the interviewer ratings and PDS were ($r = 0.41$ to $0.79$) with a median ($r = 0.70$) (Petersen et al., 1988). When examining internal consistency
with the student and the parent, the value ranges for the student’s version $\alpha = 0.67$ to 0.70) and for the parents versions were $\alpha = 0.68$ to 0.78 (Carskadon & Acebo, 1993).

This measure was chosen because some parents, school personnel, or adolescents themselves may object to a physical examination to determine pubertal status (Carskadon & Acebo, 1993; Petersen et al., 1988). This measure is suitable for a school setting, as participants will self-report their pubertal status (Patton et al., 2004; Patton et al., 2008). Reliability for the pilot study was low males ($\alpha = 0.24$) and females ($\alpha = 0.43$). Low reliability was likely due to the low number of participants in the pilot study and the low number of questions (three) for calculating reliability.

Cortisol Sampling and Analysis

Cortisol (glucocorticoid) is a hormone that is secreted as a result of stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis. When an individual perceives a stressful situation or threat, there is a response by the HPA axis which ultimately releases cortisol. This hormone releases energy stores, can enhance the immune system, and helps to increase learning and memory (Kirschbaum & Hellhammer, 1989). Collecting salivary cortisol as a physiological measure of stress is a reliable, cost effective, and efficient way to examine stress in this adolescent population (Granger, Johnson, Szanton, Out, & Schumann, 2012).

The cortisol diurnal rhythm is highest at 30 minutes after awakening, begins to decline rapidly, and is at the lowest point at approximately midnight (Hanrahan et al., 2006). When an adolescents’ cortisol diurnal rhythm is normal (higher in AM and lower in PM), there should be a negative change (negative numbers). If the change is opposite
of the normal diurnal rhythm, there should be a positive change (positive numbers). If there is a flattened or blunted rhythm, there is less than 0.01ug/dL change from morning to afternoon (Smyth et al., 1997). During adolescence there are associations between cortisol, age, and pubertal status. As the basal activity of the HPA axis increases, there is also an increase in age and sexual maturation (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). The current study will measure the cortisol diurnal rhythm to determine if the change in cortisol from morning to afternoon (cortisol diurnal rhythm) mediates the relationship between the variables of stressful life events, perceived stress, bullying, and depressive symptoms. In addition to examining the mediation relationship, the cortisol diurnal rhythm will provide data as to cortisol dysregulation (Shirtcliff & Essex, 2008; Shirtcliff et al., 2011).

There are many other activities that influence cortisol levels including smoking (Booth et al., 2008; Steptoe & Ussher, 2006), consuming caffeine (Lovallo et al., 2005), exercise (Brumby et al., 2013; McPhie & Rawana, 2012), taking medications such as corticosteroids for treatment of asthma (Granger, Hibel, Fortunato, & Kapelewski, 2009; Hibel, Granger, Kivlighan, & Blair, 2006a), and experiencing medical illness such as Cushing’s or Addison’s disease (Brown et al., 2004).

Smoking is generally associated with elevations in cortisol, and researchers have found that this is due to the nicotine exposure. When smoking was stopped, there was a decline in cortisol levels (Steptoe & Ussher, 2006). In the current study, 9th graders were asked not to smoke at least one hour before saliva samples were collected so that this would not interfere with the HPA axis response.
Caffeine has also been shown to increase the level of cortisol. In a study where individuals abstained from caffeine for five days and were then given caffeine in a pill format at varying doses, the cortisol responses increased across the day. As the amount of caffeine increased, the levels of cortisol increased (Lovallo et al., 2005). In the current study, the 9th graders were asked to refrain from drinking any caffeinated products until after the second saliva sample was collected.

Physical activity or exercise is also known to affect levels of cortisol. In a study by Brumbly and colleagues (2013) cortisol levels were decreased with regular exercise over a six month period. Although levels may acutely increase with physical exercise, the long-term effects of activity outweighed the short term with regard to improved physical and mental health. In this study, 9th graders reported physical activity; however, this was an activity that could not always be controlled due to practices for sports, dance, and physical education before, during, and after school.

Antipsychotics and hypotensive drugs can have a flattening effect on the diurnal rhythm of cortisol; however, researchers have found that antidepressants and psychostimulants have no effect on cortisol diurnal rhythm (Hibel, Granger, Cicchetti, & Rogosch, 2007). In another study, Granger and colleagues (2009) found that steroid hormones interfere with the cortisol levels due to binding with the cortisol in the body. Therefore, any 9th graders using corticosteroids for asthma or other purposes were excluded from the current study. Cortisol levels are also affected by estrogens such as birth control pills or hormones released during pregnancy and illnesses involving an elevated temperature (Granger et al., 2009), and these were also exclusion criteria for the current study.
There were no 9th grade females who self-reported pregnancy in the current study. Any 9th grader who presented with a history of a fever or physical illness was also excluded. Any medication that is considered an antimicrobial or a topical treatment in the oral cavity may also change the level of salivary cortisol; use of these medications was also on the exclusion list (Granger et al., 2009). Many other drugs (diuretics, antihistamines, barbiturates, hallucinogens, cannabis, etc.) could be listed as exclusions, but for this study, only the above medications that are more commonly used were monitored for screening out any 9th graders that would not be appropriate for the study.

In an adolescent population, cortisol levels may be different among males and females (Booth et al., 2008), and may differ according to level of pubertal status (Gunnar et al., 2009), level of socioeconomic status (Lupien et al., 2001), and level of sleep quality (Rao et al., 1996). Home and social environment as well as other major life stressors can also influence cortisol levels (Essex et al., 2011; Juster et al., 2011; McEwen, 2001).

Most adolescents will have a normal cortisol diurnal rhythm (calculated as the difference from morning to afternoon resulting in a negative number value) while others who are experiencing chronic stress may show a blunted or flattened pattern (less than 0.01ug/dL change in cortisol from morning to afternoon) or may even show an opposite diurnal rhythm depending on the level and amount of stress an individual is under and if there are internalization or externalization consequences related to the stressor (Gunnar & Vazquez, 2001; McEwen, 2003; Oskis et al., 2012; Ruttle et al., 2011). A morning and afternoon cortisol was collected in this study to measure cortisol diurnal rhythm and to determine if either morning, afternoon, or the change in cortisol from morning to
afternoon was correlated with depressive symptoms or any of the other independent variables.

The lower level sensitivity of cortisol is determined by interpolating the mean optical density minus 2 standard deviations of 10 duplicate samples at the 0µg/dL level. The minimal concentration of cortisol that can be distinguished from 0 is < 0.003µg/dL (Salimetrics, LLC, 2014). The morning and afternoon samples were analyzed by Salimetrics, LLC (2014) in duplicate with an ELISA/EIA assay, calibration range of 0.012-3.00 µg/dL and a sensitivity of < 0.003 µg/dL to determine the cortisol levels. The expected range of salivary cortisol for adolescents between the ages of 12 – 18 is 0.021 – 0.883 µg/dL for morning and 0 – 0.259 µg/dL for afternoon. The correlation between saliva and serum cortisol is $r(47) = .91, p < 0.0001$ (Salimetrics LLC, 2013). Granger and colleagues (2012) validate that integrating a measure of salivary cortisol in an adolescent population is appropriate when examining biological, behavioral, and social aspects of adolescent’s physical and mental health.

When collecting saliva, the 9th graders were asked to refrain from eating or drinking anything except water for approximately one hour before sample collection. Upon arrival to the testing room, the participants were instructed to rinse their mouth thoroughly with water 5-10 minutes before the sample was collected to minimize potential pH variability and bacterial contamination. Specimens were collected using a passive drool approach to collect in a cryo vial through a saliva collection aid for a period of three minutes while under the principal investigator’s supervision (Granger et al., 2012). The specimens of saliva were kept on ice during the time of data collection and for approximately four hours after saliva collection. Then the samples were frozen at 20°
Celsius until shipped to Salimetrics, LLC for analysis on dry ice overnight via Federal Express (Hazler et al., 2006).

Procedures for Data Collection

Once approval was obtained from the Institutional Review Board at the University of Alabama at Birmingham and the school systems superintendent and principal, the study was initiated. The data collection days were made to accommodate the school calendar. Recruitment and data collection took place during elective or non-core academic classes whenever possible. The recruitment methods from the pilot study were used to help ensure that the appropriate number of participants was recruited for this larger study.

Two research assistants were hired to assist in the current study. These assistants were required to complete the UAB Institutional Review Board Training as well as training on the specific protocol of the current study. The assistants had research experience and previous background working with adolescents in a school setting. The principal researcher and research assistants were present during the data collection process; the research assistants helped to organize the material, helped to maintain order and organization, and ensured that the 9th graders returned to the classroom when data collection was completed. The research assistants were trained as to how to answer any questions that the 9th graders had on the day of data collection (based on questions asked from the pilot study).

The principal investigator met with 9th graders in a classroom designated by the school principal and school nurse to discuss the research study and study procedures.
The 9th graders who raised their hands, showing that they were interested in participating were given the information packet including a letter to the parents explaining the purpose of the study and two parental consent forms (one to keep at home and one to return to the school). Ninth graders were instructed to have parents sign the consent form and return it to the school nurse’s office. A confidential slotted, locked, box was located on the school nurse’s office door so that adolescents could place the signed consent form in the box upon returning it to school. Each 9th grader was asked to provide a contact number so that the principal investigator could call to remind them if the consent form was not returned to the school within two weeks. The principal investigator provided a phone number for the 9th grade adolescents or the parents to call if there were questions about the current study.

The investigator met with 9th graders to collect the forms with the parental consent for participation. On that same day, the 9th graders who returned the parental consent forms were asked to complete the screening questionnaire with the investigator and then sign the assent form. The screening questionnaire was developed by the investigator and includes the following questions: What is your full name; What is your current age; Do you take any medications daily; Have you even been told that you have a diagnosis of Clinical Depression, Bipolar disorder, Addison’s disease, or Cushing’s disease; Are you pregnant (females only); Are you a smoker; Have you had an illness in the last few days that caused you to have fever where you had to stay home from school? All 9th graders who assented to participate were given a small incentive (pen, pencil, highlighter and candy at a value of $1.00 or less) once the screening form was completed regardless of whether the 9th grader qualified for the study.
On the day of data collection, 9th graders who showed interest in the current study, had been screened against inclusion and exclusion criteria, and had with parental consent and adolescent assent participated in the current study. The prospective participants were reminded again that they could decline to participate in the study without consequences. If any 9th grader refused to participate in the study on the scheduled day of data collection, the 9th grader was escorted back to the classroom and thanked for his or her interest in the study.

All 9th graders participating in the current study reported to a designated room for saliva sample collection. Saliva specimens were collected in the morning data collection prior to psychological measurements since responding to questions about stress and feelings could potentially influence cortisol levels (Preuss, Schoofs, Scholtz & Wolf, 2010). Saliva samples were collected from the 9th graders as a group in the computer room. If any 9th grader did not feel comfortable collecting the saliva sample in a group setting, that individual was allowed to collect the saliva sample in a separate room away from the others (all participants were willing to collect saliva in a group setting).

Salivary specimens were collected during either first, second, or third period class (7:30am to 10:30am) in order to standardize the time of collection, to assess cortisol diurnal rhythm (cortisol change from morning to afternoon) and to potentially control for factors that may interfere with measurement (Oskis et al., 2012; Hollenstein & Lougheed, 2013). The early morning time frame did not interfere with scheduled lunch time; however, since the adolescents were asked not to eat, smoke, or drink anything one hour before data collection time, the participants were provided a snack (chips and bottled water) after the initial data collection period.
When the 9th grade participants arrived at the computer classroom for data collection, they were asked to rinse their mouths with a small amount of water (2-4 ounces) and wait 5 to 10 minutes to re-establish a natural oral environment and pH level prior to sampling (Hanrahan et al., 2006). Each ninth grader was given a pre-numbered cryo tube and asked to insert a saliva collection aid into the cryo tube. The 9th graders were instructed to direct their saliva into the specimen tube via the specimen collection aid. The principal investigator instructed the participants to look up pictures of their favorite food on the computer while waiting for the 5-10 minutes to pass before saliva collection. The start and stop time for each 9th grader that collected saliva was documented, and as each one finished saliva collection, the principal investigator picked up each individual sample, placed it in a specimen collection box, and put the box inside a plastic bag, and then on ice. See Appendix G (Salimetrics Saliva Collection Protocol).

Once all 9th graders were completed with saliva collection, they were instructed to complete the online survey by clicking on the link located at the bottom of their individual computer screen and then answer the survey questions. Ninth graders were told to hold up their hand when completed, and the principal investigator ensured that their survey data was submitted. The principal investigator and the research assistants were present to answer questions for the 9th grade participants if assistance was needed before, during, or after completing the online survey. The 9th graders were each signed out of the first portion of the data collection period by the research assistant documenting the time each 9th grader completed the survey. Each 9th grader then selected a snack and a bottle of water before returning to class. The research assistant reminded each 9th grader what time to come back for the second data collection period.
Before the 9th graders came back for the afternoon saliva collection, the principal investigator calculated the CESD-10 scores. If any study participant reported a score greater than 9 on the CESD-10, that 9th grader’s name was given to the school nurse. The school nurse was asked to contact the 9th graders who scored greater than 9 on the CESD-10 at a time after all data collection had taken place. The nurse at that time agreed to discuss the results of the CESD-10 with the 9th grader and to help the participant make a decision as to further referral to the school counselor or to an outside referral source.

The same procedure for saliva collection took place again in the afternoon with the same 9th graders returning to the computer room for a second saliva sample at times beginning at 1pm until 3:00pm. Ninth grade participants were completed with lunch and came for the saliva collection and two other study measures during an afternoon elective class whenever possible. As the 9th graders arrived for the 2nd data collection period, they were given a small cup of water and asked to rinse their mouths to clear of any food particles or debris. While the 9th graders were waiting on others to arrive and after rinsing their mouths, each one was asked to complete The Codington’s Life Event Scale for Adolescents (CLES-A) using pencil and paper, along with an additional question: Has anything stressful happened to you today (Stressful Day)?

Once these items were completed, all participants were asked to collect their 2nd saliva sample (as a group) in a period of 3 minutes. Once the saliva samples were collected by the primary investigator and placed in a box and then on ice, each participant was provided an information sheet with phone numbers for counselors at low or no cost; these counselors were outside of the school setting. Each participant was also thanked for participating and provided with a $5.00 gift card from a local food establishment. Each
participant signed a statement agreeing that they had received the $5.00 gift card and phone numbers for counselors. The 9th graders then returned to their afternoon classes.

Data Management and Statistical Analysis Plan

Data Management

Data were collected from two high schools on separate dates over a three-month period in the Fall of 2014. To ensure integrity, data was reviewed for unintentional omissions and checked for errors in documentation by the principal researcher and research assistant at the data collection sites. Data was collected and stored on a password-protected account at Survey Gizmo. The data analysis package, Statistical Package for Social Sciences (SPSS), 2011, Version 20.0 for Windows, was used for all statistical analyses. A code book was created based on each author’s instructions and guidelines for scoring of instruments. Raw data, consent forms, and screening forms were and continue to be maintained in a locked file cabinet and accessed only by the primary investigator.

Statistical Analysis Plan

To ensure data quality, the data was examined for outliers and missing values or errors. In addition, each study variable was examined for normal distribution, homogeneity, independence, multi-collinearity, and linearity. Cronbach’s alpha coefficients were calculated to test for instrument reliability (Polit & Beck, 2012). Several statistical analyses were performed to address research questions and hypotheses with significance at the .05 level.
Research Questions, Hypotheses, and Data Analysis Plan

Research Question 1 – What are the descriptive characteristics of the measures of stressful life events, perceived stress, cortisol diurnal rhythm, and bullying in 9th grade adolescents?

Statistical Analysis – Descriptive statistics including mean, range, and standard deviation were used to examine the characteristics of the sample related to the above variables, and t-tests were used to determine the differences in the covariates (gender, race, SES, gender identity, and pubertal status) with relation to the individual independent variables and the dependent variable.

Research Question 2 – Are there correlations between stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms in 9th graders?

Hypothesis - There is a significant positive correlation between each of the variables (stressful life events, perceived stress, bullying, cortisol diurnal rhythm and depressive symptoms).

Statistical analysis – Spearman’s Rho correlational analysis was used to explore for relationships between the variables. Spearman’s Rho was used due to being an appropriate measure for the variables examined.

Research Question 3 – Do stressful life events, perceived stress, bullying, and cortisol diurnal rhythm influence depressive symptoms in 9th graders after controlling for covariates of gender, race, socioeconomic status (SES), gender identity, and pubertal status?
Hypothesis – Each independent variable (stressful life events, perceived stress, cortisol, and bullying) will separately account for a percentage of the variance in depressive symptoms. Cumulatively there will be a larger percentage related to depressive symptoms when controlling for gender, race, socioeconomic status (SES), gender identity, and pubertal status.

Statistical analysis – General Linear Models was used to determine if there was a change in $R^2$ when the different predictors are entered into the model. Predictors were entered based on a priori ideas about how the predictors go together to predict depressive symptoms.

Research Question 4 - Does cortisol diurnal rhythm mediate the relationship between stressful life events and depressive symptoms after controlling for age, gender, race, SES, gender identity, and pubertal status?

Hypothesis – Cortisol diurnal rhythm mediates the relationship between stressful life events (SLE and LCU) and depressive symptoms after controlling for gender, race, SES, gender identity, and pubertal status.

Research Question 5 – Does cortisol diurnal rhythm mediate the relationship between perceived stress and depressive symptoms after controlling for gender, race, SES, gender identity, and pubertal status?

Hypothesis – Cortisol diurnal rhythm mediates the relationship between perceived stress and depressive symptoms after controlling for gender, race, socioeconomic status (SES), gender identity, and pubertal status.
Research Question 6 – Does cortisol diurnal rhythm mediate the relationship between bullying and depressive symptoms after controlling for gender, race, SES, gender identity, and pubertal status?

Hypothesis – Cortisol diurnal rhythm mediates the relationship between bullying and depressive symptoms after controlling for age, gender, race, SES, gender identity, and pubertal status.

For research questions and hypotheses four, five, and six, the primary investigator used a mediation model analysis to examine the mediation effect of cortisol diurnal rhythm after controlling for gender, race, SES, gender identity, and pubertal status (Baron & Kenny, 1986). The following steps were completed for each one of three mediation research questions. The primary investigator used General linear models: (1) to show that the predictor variable (X) is correlated with the outcome variable (Y) (depressive symptoms), there must be an effect that may be mediated. (2) to show that the (X) is correlated with the mediating variable (M) (cortisol), the mediating variable (M) is seen as the outcome variable (Y) in the General Linear Model equation, and the predictor variable is seen as the X. (3) to show that the mediator (M) affects the dependent variable (Y), (Y) again is the outcome variable in the General Linear Model equation, and the independent variable (X) and the mediator variable (M) are predictors. The mediator and the outcome variable must be correlated because they are both caused by the initial variable (X). (4) to show that the mediator (M) completely mediates the relationship between the independent variable (X) and the dependent variable (Y), the effect of the independent variable (X) on the dependent variable (Y) controlling for the mediator variable (M) should be zero or close to zero. If all four of these steps are met,
then the data are consistent with the hypothesis that the mediation is occurring (See Figure 4).

Mediation is strongly represented when the relationship of stressful life events (Life Events and Life Change Units), perceived stress, or bullying (independent variables) to depressive symptoms (dependent variable) is zero when the mediating variable (cortisol) is included in the model (Baron & Kenny, 1986). Most phenomena in psychological analysis are caused by multiple factors, and finding a mediating relationship of zero may not be possible. Any change in significance will provide support of mediation (Baron & Kenny, 1986).

Another statistical technique that provides a more exact picture of mediation is the Sobel test (MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002; Stone & Sobel, 1990). This test is performed to determine the significance of the indirect effect of the mediator. By testing the hypothesis that no difference exists between the total effect and the direct effect, the effect is the sum of direct and the indirect effects in the mediation model. This statistical method allows for the amount of explained variance accounted for by the mediation to be estimated (Dudley, Benuzillo, & Carrico, 2004).

Summary

This chapter described the methodology for assessing the influence of stressful life events, perceived stress, bullying, and cortisol diurnal rhythm on depressive symptoms in 9th grade adolescents. The rationales for the research design, sample, setting, data collection methods, and instrumentation were addressed. The plan for protection of vulnerable subjects as well as data management and analysis were
described. As this study included physiological measures and self-report measures of stress, this combination adds strength to the research design and allows for triangulation of findings. This research adds to the scientific literature, knowledge regarding stressful life events, perceived stress, bullying, and cortisol diurnal rhythm in 9th graders with relationship to depressive symptoms.
CHAPTER FOUR

FINDINGS

This chapter will address the study findings. The purpose of the study was to examine stressful life events, perceived stress, and bullying to determine the influence of these variables individually and cumulatively on depressive symptoms after controlling for covariates (gender, race, socioeconomic status (SES), gender identity, and pubertal status). In addition, cortisol (physiological biomarker of stress) was examined to determine if the cortisol diurnal rhythm had a mediating relationship between each of the independent variables (stressful life events (SLE, LCU), perceived stress (PSS-10), bullying (PECK), and the dependent variable, depressive symptoms (CESD-10) after controlling for the aforementioned covariates.

In this chapter the following study findings will be discussed: reliability of study instruments, sample and setting characteristics, and descriptive statistics for each variable including: Cortisol (AM, PM), Stressful Day, and Smoking Status. A summary of findings is discussed for each research question and its accompanying hypothesis. Additional analyses were conducted on questions posed to adolescents at the end of the online survey including: living arrangements, was anything upsetting in the survey, and interest in participating in future research. Analyses related to correlations of the independent variables with the dependent variable (CESD-10), and cortisol diurnal rhythm are discussed.
Instrument Reliability

Reliability statistics measuring internal consistency were completed for the following measures: Center for Epidemiological Studies Depression Survey-10 (CESD-10); Coddington Life Event Scale – Adolescents (CLES-A); Perceived Stress Scale (PSS-10); Personal Experiences Checklist (PECK); and the Self-Rating Scale for Pubertal Development. The coefficient alpha is an estimate of the extent that the different parts of the instrument are consistently measuring an underlying attribute. Cronbach’s alpha of 0.8 – 0.9 indicates a strong internal consistency within the items of the measure (Polit & Beck, 2012).

The stressful life event survey had a fair internal consistency (\( \alpha = 0.76 \)), and the remaining scales had strong internal consistency (\( \alpha = 0.87 \) to 0.94) with the exception of the Self-Rating Scale for Pubertal Development. Reliability was calculated by gender for the Self-Rating Scale for Pubertal Development because the measure consisted of different questions for males as compared to females, and reliability was calculated from five questions. Inadequate reliability was found for both males (\( \alpha = 0.66 \)) and females (\( \alpha = 0.44 \)). Results are summarized on Table 2.
Table 2

*Instrument Reliability (N = 143)*

<table>
<thead>
<tr>
<th>Instrument</th>
<th># Items</th>
<th>α coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center for Epidemiological Studies Depression Survey-10 (CESD-10)</td>
<td>10</td>
<td>0.86</td>
</tr>
<tr>
<td>Coddington Life Event Survey-Adolescents (CLES-A)</td>
<td>50</td>
<td>0.76</td>
</tr>
<tr>
<td>Perceived Stress Scale-10 (PSS-10)</td>
<td>10</td>
<td>0.87</td>
</tr>
<tr>
<td>Personal Experiences Checklist</td>
<td>32</td>
<td>0.94</td>
</tr>
<tr>
<td>Self-Rating Scale for Pubertal Development-Male</td>
<td>5</td>
<td>0.66*</td>
</tr>
<tr>
<td>Self-rating Scale for Pubertal Development- Female</td>
<td>5</td>
<td>0.44**</td>
</tr>
</tbody>
</table>

*Note.* *Males Pubertal (49 cases), **Females Pubertal (94 cases).*

Sample, Setting, and Characteristics

A convenience sample of adolescents (n = 143) aged 14-16 participated in the study at two different high schools in a Southern state. Adolescents in the 9th grade from School A were recruited during an elective class in the months of August, September, and November. Adolescents in the 9th grade from School B were recruited at a freshmen assembly during the month of October. School A had a total of 300 freshmen (9th grade) adolescents, and School B had a total of 102 freshmen (9th grade) adolescents. The combined total of adolescents was n = 402, 9th graders. Recruitment consisted of providing packets with a letter to the parents; refusal form; a consent form to sign and return; and a consent form to keep at home. These packets were distributed to adolescents who expressed an interest in the study. Approximately 250 packets were provided at School A and
150 packets were provided at School B; this amount included extra packets that were provided when requested by 9th graders who reported misplacing their packets. The total number of packets provided to potential participants was approximately 400. The total number of 9th graders recruited \((n = 260)\), screened \((n = 152)\), parent refusals \((n = 31)\), 9th graders with no interest in participating \((n = 72)\), and total response rate \((n = 143, 55\%)\) is summarized on Table 3.

The a priori power analysis for participation in this study was based on a medium effect size \((R^2 = 0.13)\) for a general linear model. Using G-Power analysis (Faul et al., 2000), a sample size of 136 adolescents was determined to be an appropriate sample size for this study. Enrollment at the time the study began was \(n = 144\). The final sample size was \(n = 143\) adolescents. Data from one participant was not used due to not meeting the inclusion criteria.

Table 3

<table>
<thead>
<tr>
<th>Total 9th grade</th>
<th>Recruited</th>
<th>Screened</th>
<th>Parents refusal</th>
<th>No Interest</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>(n) (%)</td>
<td>(n) (%)</td>
<td>(n) (%)</td>
<td>(n) (%)</td>
<td>(n) (%)</td>
</tr>
<tr>
<td>402</td>
<td>260 (65)</td>
<td>152 (59)</td>
<td>31 (12)</td>
<td>72 (27)</td>
<td>143 (55)</td>
</tr>
</tbody>
</table>

Socio-Demographic Characteristics of the Sample

The study sample was predominantly female \((66\%)\), age 14 years \((78.3\%)\), and White \((76\%)\). Gender identity categories as self-reported by study adolescents were heterosexual \((n = 122, 85.3\%)\), homosexual \((n = 2, 1.4\%)\), bisexual \((n = 13,\)
9.1%), and unsure (n = 6, 4.2%). The National School Lunch Program (NSLP) was used as a proxy to measure socioeconomic status in this study. The majority of the sample either received free or reduced-cost lunch (n = 68, 47.6%), or paid full price for lunch (n = 57, 39.9%), with the minority of adolescents reporting bringing a lunch from home (n = 18, 12.6%). Socio-demographic characteristics are summarized on Table 4.

Table 4

Socio-Demographic Characteristics of the Sample (N = 143)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Category</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td>14 years</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>15 years</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>16 years</td>
<td>5</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>94</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>9</td>
</tr>
<tr>
<td>Gender identity</td>
<td>Heterosexual</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Homosexual</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bisexual</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Unsure</td>
<td>6</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>Free/reduced</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Full pay</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>18</td>
</tr>
</tbody>
</table>
Health Variables

**Pubertal status**

Pubertal status was measured using the Self-Rating Scale for Pubertal Development (PDS), a five-item instrument with five ordinal Likert-type scale responses (Carskadon & Acebo, 1993). Total pubertal status scores are determined for males based on responses to questions about body hair growth, voice change, and facial hair growth. Female pubertal status was determined by answers to questions about body hair growth, breast development, and menarche. The majority of males were mid-to late-pubertal status, and the females were late or post-pubertal status. A summary of pubertal status can be found on Table 5.

**Smoking status**

During the first data collection session, adolescents were asked a question about smoking (Are you a smoker? yes/no). Only 4.2% of the adolescents were self-reported smokers. A summary of smoking status can be found on Table 5.

Table 5

*Health Variables: Pubertal status and Smoking Status (N = 143)*

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>$\bar{x}$</th>
<th>$SD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubertal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0-12</td>
<td>8.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Female</td>
<td>1-12</td>
<td>9.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>(4.2)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>137</td>
<td>(95.8)</td>
<td></td>
</tr>
</tbody>
</table>
Descriptive Statistics: Cortisol (AM, PM, Diurnal Rhythm), and Stressful Day

Descriptive statistics including mean, range, and standard deviation were used to examine cortisol levels and Stressful Day. Cortisol levels were measured as morning (AM), afternoon (PM), and cortisol diurnal rhythm.

**Cortisol**

Salivary specimens for cortisol were collected in the morning during 1\textsuperscript{st}, 2\textsuperscript{nd}, or 3\textsuperscript{rd} period and again in the afternoon during 5\textsuperscript{th}, 6\textsuperscript{th}, or 7\textsuperscript{th} period. Saliva samples to measure cortisol AM and PM were collected over a period of three minutes with the majority of adolescents; however, there were a few adolescents that took less than 3 minutes to collect the required amount of saliva and a few adolescents that took more than 3 minutes (cortisol AM - $\bar{x} = 1 \text{ min. to 12 min.}$, $SD = 3 \text{ min.}$, cortisol PM - $\bar{x} = 1 \text{ min. to 14 min.}$, $SD = 3 \text{ min.}$). The time between cortisol AM and PM saliva collection time ranged from 1 hour, 30 minutes to 6 hours, 53 minutes ($\bar{x} = 4 \text{ hrs., 40 min, } SD = 1 \text{ hr., 9 min.}$).

The expected range of cortisol level for adolescents in the AM is $0.021\mu g/dl - 0.883 \mu g/dl$, and the range for the cortisol level in the PM is $0 - 0.259 \mu g/dl$ (Salimetrics, LLC, 2013). The levels for cortisol AM and cortisol PM were in the expected ranges for adolescents. Cortisol diurnal rhythm was measured as the change in the cortisol AM to the cortisol PM. This change is reflected as a negative number value due to the decline from morning to afternoon). The results for descriptive statistics of cortisol levels are summarized on Table 6.
Table 6

Descriptive Statistics: Cortisol AM, Cortisol PM, and Cortisol Diurnal Rhythm
(N = 143)

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>( \bar{x} )</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol AM</td>
<td>0.042-0.754</td>
<td>0.197</td>
<td>0.134</td>
</tr>
<tr>
<td>Cortisol PM</td>
<td>0.019-0.624</td>
<td>0.125</td>
<td>0.081</td>
</tr>
<tr>
<td>Cortisol diurnal rhythm</td>
<td>-0.66–0.53</td>
<td>-0.07</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Note. Cortisol levels in \( \mu g/dL \)

Cortisol levels are expected to be higher in the AM and drop significantly in the PM (Granger, et al., 2012). Outliers in cortisol AM and PM levels were noted, and there was no linear trend, as displayed on the scatterplot (Figure 4).

Figure 4

Scatterplot – X – Cortisol AM, Y – Cortisol PM
Cortisol diurnal rhythm is found by determining the change from AM cortisol levels to PM cortisol levels. This change is seen as a negative change (negative numbers) for those in the normal diurnal rhythm category. Those who have a positive change are in the category of opposite diurnal rhythm. Those who have less than 0.01 \( \text{ug.dL} \) change from AM to PM were in the flattened or blunted category. These data points are seen on Figure 5 scatterplot. Adolescents who were in normal diurnal rhythm category had the longest time period between AM and PM saliva collection and are seen on the far right side of the scatterplot (\( \bar{x} = 4:55 \text{min.}, \ SD = 1.05 \text{min.} \)). The adolescents who had less than 0.01 change from morning to afternoon had the middle range of time (\( \bar{x} = 4:23 \text{min.}, \ SD = 1:04 \text{min.} \)) and are seen in the middle of the scatterplot centered around the point of 0 or no significant change in cortisol from morning to afternoon. Adolescents who had an opposite cortisol diurnal rhythm are represented in lowest time range (\( \bar{x} = 4:08 \text{SD} (1:12) \)) and are seen on the far left side of the scatterplot.
Figure 5

Scatterplot: X – Time between AM and PM saliva collection, Y- cortisol diurnal rhythm

Stressful Day

At the end of the 2nd data collection period, adolescents were asked the following question: “Did anything stressful occur today?” (Stressful Day). More than one half of the sample (n = 82, 57.3%) reported that no stressful event occurred, and less than one half responded that there was a stressful event that occurred (n = 61, 42.7%). Respondents who answered “yes” to a Stressful Day were asked to describe the event(s). Content analysis was used to categorize the participant responses. Five categories were identified: family issues; peer issues,
school issues, personal or physical issues, and romantic issues. Results are summarized on Table 7.

School issues were identified as the stressor by the majority of the adolescents (55.7%), and included responses such as “I failed a test today” or “I left my binder at home this morning.” Peer issues were identified by 14.8% of adolescents and included responses such as “I was left out of a group during the break today.” Family issues were identified by 13% of the adolescents and included responses such as “My mom was yelling at me this morning.” Romantic issues were identified by 13% of adolescents and included responses such as “I broke up with my girlfriend today.” Personal or physical issues were identified by 11.5% of adolescents and included responses such as “I had a headache from not being able to hear the teacher over all the talking” or “my baby was crying while I was trying to get ready for school.”

Table 7

*Descriptive Statistics: Stressful day - Yes or No, and Categories of Issues/Events*

<table>
<thead>
<tr>
<th>Issues/Event</th>
<th>Stressful Day</th>
<th></th>
<th>Stressful Day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>34</td>
<td>55.7</td>
<td>82</td>
<td>57.3</td>
</tr>
<tr>
<td>Peer</td>
<td>9</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>8</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romantic</td>
<td>8</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal/Physical</td>
<td>7</td>
<td>11.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bivariate relationships between the covariates (gender, race, SES, gender identity, pubertal status) and cortisol (AM, PM.) were examined using t-tests. To examine the relationships between the variables of Stressful Day, Smoking Status and covariates, cross tabulations and Pearson’s Chi Square tests were conducted. Results are summarized on Tables 8, 9, 10, and 11.

Due to the small cell sizes for the categories of race, SES, gender identity, and pubertal status, these variables were collapsed into 2 categories. Race was categorized as White and Black/other, SES was categorized as Free/reduced-cost lunch and Full-price lunch/other, gender identity was categorized as heterosexual and homosexual/bisexual/unsure. When examining pubertal status categories, Peterson and colleagues (1988) discuss five levels of puberty: pre-pubertal, early-pubertal, mid-pubertal, late-pubertal, and post-pubertal. Because there were no adolescents at pre-pubertal or early status, for the current study, pubertal status was collapsed into two categories for analysis purposes. These categories were early-to mid-pubertal status, and late-to post-pubertal status.

Gender

Female adolescents had significantly higher cortisol AM levels as compared to male adolescents ($t (138.19), = -3.49, p = 0.001$). Though there were no significant differences in the cortisol PM by gender ($t (141) = -1.164, p = 0.247$). More female adolescents self-reported a stressful day than males; however, these differences were non-significant ($\chi^2 = 0.103, df = 1, p = 0.748$).
Although five out of the six self-reported smokers were females, the differences between males and females were also non-significant ($\chi^2 = 0.861, df = 1, p = 0.353$).

**Race**

Black adolescents had higher cortisol AM levels than White adolescents; however, these differences were not significant ($t (141) = -0.669, p = 0.504$). Cortisol PM levels were approximately the same between Black and White adolescents; therefore, there were no significant differences by race ($t (141) = -0.481, p = 0.631$). More White adolescents reported having a stressful day than Black/other adolescents; yet, these differences were not significant ($\chi^2 = 2.389, df = 1, p = 0.122$). Although five out of the six smokers in the sample were White, the difference in race was not significant ($\chi^2 = 0.207, df = 1, p = 0.649$).

**Socioeconomic status**

Cortisol AM levels ($t (141) = -1.170, p = 0.244$) and cortisol PM levels ($t (141) = -0.317, p = 0.752$) were slightly higher in adolescents who reported paying the full price for lunch or in those who brought lunch from home as compared to the adolescents who reported receiving free/reduced-cost lunch; however, these differences were non-significant. More adolescents receiving a full pay lunch or lunch brought from home reported a stressful day as compared to those adolescents receiving free/reduced-cost lunch; yet again, these differences were not significant ($\chi^2 = 0.113, df = 1, p = 0.737$). Although four out of six smokers in the sample received free or reduced-cost lunch, there were no
significant differences between those who paid full price for lunch or brought lunch from home \((\chi^2 = 0.917, df = 1, p = 0.338)\).

*Gender identity*

Homosexual/bisexual/unsure adolescents had slightly higher cortisol AM than heterosexual adolescents; however, these differences were non-significant \((t(141) = -0.726, p = 0.469)\). The opposite was true for the cortisol PM with heterosexuals having slightly higher cortisol PM levels; but again, these were non-significant differences \((t(141) = 0.839, p = 0.403)\). More self-reported homosexual/bisexuals and those who were unsure of their gender identity had a stressful day as compared to those who were heterosexual; however, these differences were non-significant \((\chi^2 = 0.951, df = 1, p = 0.329)\). There were significant gender identity differences between the smokers and the nonsmokers. Four of the smokers self-reported their gender identity as homosexual/bisexual, and those who were unsure of their gender identity; whereas, only two of the smokers self-reported heterosexuality \((\chi^2 = 13.507, df = 1, p < 0.001)\).

*Pubertal Status*

Adolescents who self-reported mid-pubertal status had significantly lower AM levels of cortisol as compared to adolescents who self-reported late to post-pubertal status \((t(130.8) = -2.745, p = 0.007)\). The same was true of the cortisol PM levels, in that those adolescents who were early to mid-pubertal status had lower cortisol than the late to post-pubertal status adolescents; however, the differences were not significant \((t(141), 0.730, p = 0.466)\). More adolescents who self-reported late to post pubertal status also reported a stressful day as
compared to those adolescents who were early to mid-pubertal status; yet again, these differences were non-significant ($\chi^2 = 1.204, df = 1, p = 0.272$). Although five out of the six smokers were in the late to post-pubertal category, the differences were non-significant in comparison to those who were early to mid-pubertal status ($\chi^2 = 0.745, df = 1, p = 0.388$) due to the small cell sizes.

Table 8

*Independent Samples t-test: Mean & SD Cortisol AM by Covariates*

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Category</th>
<th>n</th>
<th>$\bar{x}$</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>49</td>
<td>0.11</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>94</td>
<td>0.14*</td>
<td>0.008</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>108</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Black/other</td>
<td>35</td>
<td>0.21</td>
<td>0.16</td>
</tr>
<tr>
<td>SES</td>
<td>Free/reduced</td>
<td>68</td>
<td>0.18</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Full pay/other</td>
<td>75</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Gender ID</td>
<td>Heterosexual</td>
<td>122</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Homosexual/other</td>
<td>21</td>
<td>0.21</td>
<td>0.17</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>Early to mid-puberty</td>
<td>47</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Late to post puberty</td>
<td>96</td>
<td>0.22</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Note. * $p < 0.05$
Table 9

*Independent Samples t-test: Mean & SD of Cortisol PM by Covariates*

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Categories</th>
<th>n</th>
<th>( \bar{x} )</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>49</td>
<td>0.11</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>94</td>
<td>0.13</td>
<td>0.008</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>108</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Black/other</td>
<td>35</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>SES</td>
<td>Free/reduced</td>
<td>68</td>
<td>0.12</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Full pay/other</td>
<td>75</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender ID</td>
<td>Heterosexual</td>
<td>122</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Homosexual/other</td>
<td>21</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>Early to mid- puberty</td>
<td>47</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Late to post puberty</td>
<td>96</td>
<td>0.21</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Note.* No significant differences in covariates and cortisol PM levels

Table 10

*Pearson’s Chi Square Cross Tabulations: Stressful Day by Covariates*

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Categories</th>
<th>n</th>
<th>% No</th>
<th>% Yes</th>
</tr>
</thead>
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<tr>
<td>Gender</td>
<td>Male</td>
<td>49</td>
<td>59.2</td>
<td>40.8</td>
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<td></td>
<td>Female</td>
<td>94</td>
<td>56.4</td>
<td>43.6</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>108</td>
<td>53.7</td>
<td>46.3</td>
</tr>
<tr>
<td></td>
<td>Black/other</td>
<td>35</td>
<td>68.6</td>
<td>31.4</td>
</tr>
<tr>
<td>SES</td>
<td>Free/reduced</td>
<td>68</td>
<td>55.9</td>
<td>44.1</td>
</tr>
<tr>
<td></td>
<td>Full pay/other</td>
<td>75</td>
<td>58.7</td>
<td>41.3</td>
</tr>
<tr>
<td>Gender ID</td>
<td>Heterosexual</td>
<td>122</td>
<td>59.0</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>Homosexual/other</td>
<td>21</td>
<td>47.6</td>
<td>52.4</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>Early to mid- puberty</td>
<td>47</td>
<td>63.8</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>Late to post puberty</td>
<td>96</td>
<td>54.2</td>
<td>45.8</td>
</tr>
</tbody>
</table>

*Note.* No significant differences in covariates and cortisol PM levels
Table 11

Pearson’s Chi Square Cross Tabulations: Smoking Status by Covariates

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Categories</th>
<th>n</th>
<th>% No</th>
<th>% Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>49</td>
<td>98.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>94</td>
<td>94.7</td>
<td>5.3</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>108</td>
<td>95.4</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Black/other</td>
<td>35</td>
<td>97.1</td>
<td>2.9</td>
</tr>
<tr>
<td>SES</td>
<td>Free/reduced</td>
<td>68</td>
<td>94.1</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Full pay/other</td>
<td>75</td>
<td>97.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Gender ID</td>
<td>Heterosexual</td>
<td>122</td>
<td>98.4</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Homosexual/other</td>
<td>21</td>
<td>81.0</td>
<td>19.0*</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>Early to mid-puberty</td>
<td>47</td>
<td>97.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Late to post puberty</td>
<td>96</td>
<td>94.8</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Note. *p < 0.05

Bivariate Relationships: Stressful Day and Smoking Status by Cortisol (AM and PM), Independent Variables, and Dependent Variable

To examine the relationships between the variables, cortisol (AM, PM), independent variables (stressful life events [SLE], life change units [LCU], perceived stress [PSS-10], bullying [PECK]), and the dependent variable (depressive symptoms [CESD-10]), Stressful Day (Did anything stressful occur today?) and Smoking Status, t-tests were conducted. Results are summarized on Table 12 and 13.

Adolescents who reported a stressful day reported more stressful life events (SLE scores) as compared to those who did not report a stressful day, though these differences were not significant ($t (141) = -1.805, p = 0.079$).
However, adolescents who reported a stressful day had significantly higher LCU scores as compared to those who did not report a stressful day ($t (100.34) = -2.549, p = 0.012$).

Adolescents who reported a stressful day had higher perceived stress scores (PSS-10) as compared to those who did not report a stressful day, however, these differences were not significant ($t (141) = -1.841, p = 0.068$). The same held true for bullying scores with the differences being higher but not significant between adolescents who reported a stressful day as compared to those who did not report a stressful day ($t (141) = -1.212, p = 0.228$).

Verbal/relational bullying scores were higher in those who reported a stressful day as those who did not report a stressful day; however, these differences were not significant ($t (141) = -1.571, p = 0.119$). Cyberbullying scores were also slightly higher in those who had a stressful day as compared to those who did not have a stressful day, but again, these differences were non-significant ($t (139) = -0.677, p = 0.500$). Cultural bullying scores were higher in those who did not have a stressful day, but these differences were not significant ($t (130.7) = 0.885, p = 0.378$). Physical bullying scores were higher in those who answered yes to having a stressful day as compared to those who did not have a stressful day; but again, these differences were not significant ($t (139) = -0.818, p = 0.415$).

Adolescents who had a stressful day had slightly higher CESD-10 scores than those adolescents who did not have a stressful day; however, there was no
significant difference \((t(141) = -0.734, p = 0.464)\). Cortisol AM levels were slightly higher for those who did not report a stressful day; but again, these differences were non-significant \((t(141) = 0.554, p = 0.580)\). The opposite was true for cortisol PM levels in that those who had a stressful day had slightly higher levels than those who did not report a stressful day, but again, these were non-significant differences \((t(141) = -0.082, p = 0.935)\). Results are summarized on Table 12.

Table 12

*Independent Samples t-tests: Mean and SD of Stressful Day by Independent, Dependent, Cortisol AM, PM*

| Variable | Stressful Day- Yes 
\((n = 61)\) | Stressful Day- No 
\((n = 82)\) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x} (SD))</td>
<td>(\bar{x} (SD))</td>
</tr>
<tr>
<td>SLE</td>
<td>15.9(11.1)</td>
<td>12.7 (9.7)</td>
</tr>
<tr>
<td>LCU</td>
<td>482(354)*</td>
<td>348(243)</td>
</tr>
<tr>
<td>PSS-10</td>
<td>20(7)</td>
<td>18(7)</td>
</tr>
<tr>
<td>PECK</td>
<td>17(15)</td>
<td>14(15)</td>
</tr>
<tr>
<td>CESD-10</td>
<td>9(7)</td>
<td>9(6)</td>
</tr>
<tr>
<td>Cortisol AM</td>
<td>0.19(0.13)</td>
<td>0.20(0.13)</td>
</tr>
<tr>
<td>Cortisol PM</td>
<td>0.13(0.09)</td>
<td>0.12(0.07)</td>
</tr>
</tbody>
</table>

*Note.* \(p < 0.05\)
Independent samples t-tests were computed between those who answered “Yes” to the smoking status question as compared to those who answered “No” to the smoking status question. Those who answered yes to smoking status had significantly higher stressful life event scores (SLE) as compared to those who answered no to the smoking status question ($t(141) = 2.041, p = 0.043$). The same was true for the life change units (LCU) with smoking status answer being yes having significantly higher LCU scores than smoking status answer being no ($t(141) = 2.98, p < 0.003$). Although the mean scores and standard deviation scores were higher on the perceived stress scale (PSS-10) ($t(141) = 1.63, p = 0.104$) and the bullying scale (PECK), ($t(141) = 1.78, p = 0.077$), these differences were not significant.

Verbal-relational bullying scores were significantly higher for those who answered Yes to the smoking status question as compared to those who answered No ($t(139) = 2.000, p = 0.045$). Cyberbullying scores were also significantly higher for those who answered Yes to the smoking status question as compared to those who answered No ($t(141) = 0.200, p = 0.047$). Cultural bullying scores were approximately equal between those who answered yes to the smoking status question as compared to those who answered No ($t(141) = -0.142, p = 0.887$). The same results were true of physical bullying with non-significant differences; however, those who answered yes to the smoking status question were bullied physically slightly more than those who answered no to the smoking status question ($t(139) = 0.669, p = 0.504$).
Adolescents who answered “Yes” to the smoking status question had significantly higher depressive symptoms (CESD-10) as compared to those who answered “No” ($t_{(141)} = 2.132, p = 0.035$). Cortisol AM levels were higher in those who answered Yes to the smoking status question as compared to those who answered No; however, these differences were non-significant ($t_{(141)} = -0.861, p = 0.391$). Cortisol PM levels for those who answered Yes to the smoking status question were slightly higher than those who answered No to the smoking status question; however, these differences were again, non-significant ($t_{(141)} = 0.631, p = 0.529$). Results are summarized on Table 13.

Table 13

*Independent Samples t-tests: Mean and SD of Smoking Status with Independent, Dependent, Cortisol AM, PM*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smoker-Yes ($n = 6$)</th>
<th>Smoker-No ($n = 137$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ ($SD$)</td>
<td>$\bar{x}$ ($SD$)</td>
</tr>
<tr>
<td>SLE</td>
<td>22.5(7.2)*</td>
<td>13.7(10.4)</td>
</tr>
<tr>
<td>LCU</td>
<td>756(263)*</td>
<td>390(295)</td>
</tr>
<tr>
<td>PSS-10</td>
<td>23.7(9.5)</td>
<td>18.7(7.1)</td>
</tr>
<tr>
<td>PECK</td>
<td>25.8(18.6)</td>
<td>14.7(14.8)</td>
</tr>
<tr>
<td>CESD-10</td>
<td>14.3(9.5)*</td>
<td>8.7(6.1)</td>
</tr>
<tr>
<td>Cortisol AM</td>
<td>0.15(0.09)</td>
<td>0.19(0.13)</td>
</tr>
<tr>
<td>Cortisol PM</td>
<td>0.14(0.10)</td>
<td>0.12(0.08)</td>
</tr>
</tbody>
</table>

*Note.* *$p < 0.05$*
Findings Related to Research Questions

Research Question 1

What are the descriptive characteristics of the variables of stressful life events, perceived stress, cortisol diurnal rhythm, bullying, and depressive symptoms in 9th grade adolescents?

Descriptive statistics including mean, range, and standard deviation were used to examine the characteristics of the sample related to the above variables. T-tests were computed to determine the differences in covariates (gender, race, SES, gender identity, and pubertal status) with relation to the individual independent variables (SLE, LCU, PSS-10, and PECK) and the dependent variable (CESD-10). The descriptive statistics between the independent variables of stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms were examined as well as the types of bullying. Findings are summarized on Table 14.

Stressful life events

Stressful life events were measured using the self-report Coddington Life Event Scale – Adolescents (CLES-A), with scores calculated as the number of stressful life events in the last year (SLE), and the Life Change Units (LCU) score indicating the amount of social readjustment after the adolescent experienced the stressful life event. The LCU is a weighted score that is summed for each individual SLE occurring. The events are documented as happening in the last 0 – 3 months, 4 – 6 months, 7 – 9 months, and 10 – 12 months. The more recent events have a higher weight than later events. The higher scores indicate higher
weighting of the individual stressful life events. A score of 170 is the normative high score for the LCU. This means that on average, 75% of adolescents (ages 14 to 16) scored below 170 (Coddington, 1999).

In the current study, the mean SLE score was 14 ($SD = 10$). Adolescents reported on average, 14 individual events that caused stress over the last year (SLE). The mean LCU score was 406 ($SD = 302$), with scores ranging from 0 to 1691. The majority of adolescents ($79.7\%, n = 114$) scored greater than the normative high score range from 170 to 1691 and only $20.3\% (n = 29)$ scored less than the normative scores (ranging from 0 to 170).

Perceived stress

The Perceived Stress Scale (PSS-10) is a measure of perceived stress in an adolescent. PSS-10 scores can range from 0 to 40 with the higher stress score indicating greater stress. There was no “cut off” score provided for the PSS-10 in the literature, only comparisons between individuals in the study sample (Cohen, 2014; Cohen, 1986). Therefore, the distribution of the PSS-10 scores was examined. The mean score was 19 ($SD = 7$). Few adolescents ($n = 4, 2.8\%$) scored from 31 to 40 on the PSS-10. Few adolescents ($n = 19, 13.3\%$) scored in the highest range of the PSS-10. However, the majority of the adolescents ($n = 120, 83.9\%$) scored in the middle range (score of 11 to 30). The mean PSS-10 score in this study was 19 ($SD = 7$) with scores ranging from 1 to 40.

Cortisol diurnal rhythm

Cortisol diurnal rhythm was measured by the change in the cortisol AM to cortisol PM (reflective in a negative value). Cortisol diurnal rhythm levels ranged
between -0.66 µg/dL to 0.53 µg/dL with a mean of 0.07 (SD = 0.15). Cortisol AM levels are expected to be higher and drop significantly in the PM (Granger et al., 2012). In this study, over half of the adolescents (n = 92, 64.3%) had a diurnal rhythm with cortisol levels that were higher in the AM and lower in the PM. However, there was a small group (n = 18, 12.6%) that had a flattened diurnal rhythm (less than 0.01 µg/dL change from morning to afternoon) and another slightly larger group (n = 33, 23%) that had an opposite diurnal rhythm, meaning that the cortisol was low in the AM and high in the PM.

There were significant differences in cortisol diurnal rhythm patterns by gender (χ² = 7.296, df = 2, p = 0.026). Out of all females, 70.2% had a normal diurnal rhythm. Out of all males, 53.1% had a normal diurnal rhythm.

When examining results from adolescents who had an opposite diurnal rhythm (n = 33), the cortisol levels increased from morning to afternoon from 0.02 µg/dL to 0.53 µg/dL. There was approximately the same percentage of males (24.5%) and females (22.3%) in this category. When examining those adolescents who had a flattened diurnal rhythm (n = 18), there was a higher percentage of males (22.4%) in this category than females (7.4%).

**Bullying**

The Personal Experiences Checklist (PECK) is a self-report measure of four different types of bullying (verbal/relational, physical, cyber, and cultural bullying). The instrument is scored with a range of 0 to 4 with the following values: 0 (never), 1 (rarely), 2 (sometimes), and 3 (most days), and 4 (every day), with higher scores indicating greater bullying (total possible score of 128). The
PECK survey had no predetermined “cut point” or significant bullying score; however, a level of 2 to 3 times of being bullied per month was recommended as the lower bound cut off from the Olweus Bullying Victim Questionnaire (Solberg & Olweus, 2003) In this study the mean PECK score was 15.13 ($SD = 15.14$) with a range of 0 to 98. Subscale scores were as follows: verbal/relational bullying scores ($\bar{x} = 8$, $SD = 8$, range 0-38) were higher than physical bullying ($\bar{x} = 2$, $SD = 8$, range, 0-21), cyberbullying ($\bar{x} = 2$, $SD = 3$, range, 0-28) or cultural bullying ($\bar{x} = 2$, $SD = 2$, range, 0-15).

**Depressive symptoms**

The Center for Epidemiological Studies Depression Survey-10 (CESD-10) was the instrument used to measure depressive symptoms. This 10-item Likert scale ranges from 0 (rarely) to 3 (all the time), yielding a total score of 0 to 30. A score of 10 or greater indicated a need for referral (clinically meaningful depressive symptoms) (Raffaelli et al., 2013). In this study, the CESD-10 scores ranged from 0 to 29, with a mean score of 9 ($SD = 6$). The majority of adolescents (60.8%) did not have clinically significant depressive symptoms (score of 9 or less); however, 39.2% had scores of 10-30. Any participant who scored greater than 9 on the CESD-10 was referred to the school nurse who would call in the adolescent individually for a consultation about the CESD-10 score to further explore that adolescent’s mental status.
Table 14

Descriptive Characteristics of Independent Variables, Dependent Variables, Cortisol Diurnal Rhythm, and Types of Bullying (N = 143)

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\bar{x}$</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>14</td>
<td>10</td>
<td>0-79</td>
</tr>
<tr>
<td>LCU</td>
<td>406</td>
<td>302</td>
<td>0-1681</td>
</tr>
<tr>
<td>PSS-10</td>
<td>19</td>
<td>7</td>
<td>1-39</td>
</tr>
<tr>
<td>CESD-10</td>
<td>9</td>
<td>6</td>
<td>0-29</td>
</tr>
<tr>
<td>Cortisol diurnal rhythm</td>
<td>-0.07</td>
<td>0.15</td>
<td>-0.66 - 0.53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\bar{x}$</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PECK</td>
<td>15</td>
<td>15</td>
<td>0-98</td>
</tr>
</tbody>
</table>

Types of bullying

<table>
<thead>
<tr>
<th>Type</th>
<th>$\bar{x}$</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal/rel.</td>
<td>8</td>
<td>8</td>
<td>0-38</td>
</tr>
<tr>
<td>Physical</td>
<td>2</td>
<td>8</td>
<td>0-21</td>
</tr>
<tr>
<td>Cyber</td>
<td>2</td>
<td>3</td>
<td>0-28</td>
</tr>
<tr>
<td>Cultural</td>
<td>2</td>
<td>2</td>
<td>0-15</td>
</tr>
</tbody>
</table>

Bivariate Relationships between Independent, Dependent Variables, Cortisol Diurnal Rhythm, Bullying Types and Covariates

Relationships were examined between the covariates (gender, race, SES, gender identity, and pubertal status), the mediating variable (cortisol diurnal rhythm), and the independent variables (stressful life events, perceived stress, and bullying), and the dependent variable (depressive symptoms). T-tests were conducted to determine if there were significant differences between the groups.
within the covariates with regard to the independent variables. Race, SES, gender identity, and pubertal status were collapsed into two categories for appropriate analysis (see previous explanation in descriptive statistics). In addition, covariates were compared by types of bullying (verbal/relational, physical, cyber, and cultural). Results are summarized on Tables 15, 16, and 17.

**Gender**

With regard to gender as a covariate, females had higher SLE scores than males; however, the differences were not significant ($t (141) = -0.784, p = 0.434$). Females also had higher LCU scores than males, but again, the differences were non-significant ($t (141) = -0.806, p = 0.425$). There were significant gender differences in PSS-10 scores ($t (141) = -2.49, p = 0.014$) with females reporting higher levels of perceived stress than males. Males had higher PECK scores than females; however, these differences were non-significant ($t (67.98) = 1.359, p = 0.182$).

Males adolescents reported significantly higher physical bullying as compared to females ($t (58.72) = 3.686, p < 0.001$). Higher scores, though not significant, for males were found for verbal/relational ($t (65.413) = 0.281, p = 0.706$). Males also had higher cyberbullying and cultural bullying scores than females; but again, these differences were non-significant. Females had higher CESD-10 scores than males; however, these differences were non-significant ($t (141) = -0.321, p = 0.749$). Females had a significantly larger cortisol change from AM to PM (cortisol diurnal rhythm) than males, equal variances not assumed ($t (139.265) = 2.408, p = 0.017$).
With regard to race, White adolescents had higher SLE scores than Black/other adolescents; however, these differences were non-significant ($t(141) = 1.086, p = 0.279$). White adolescents had higher LCU scores than Black/other adolescents; but again, these differences were non-significant ($t(141) = 0.519, p = 0.473$). White adolescents had approximately the same PSS-10 scores as Black/other adolescents; therefore, there was no significant difference ($t(141) = 0.221, p = 0.825$). Black/other adolescents had higher PECK scores than White adolescents; however, these differences were also non-significant ($t(141) = 0.675, p = 0.822$).

Adolescents who self-reported as Black/other had significantly higher cultural bullying scores than White adolescents, equal variances not assumed ($t(40.793) = -2.758, p = 0.044$). White adolescents had higher verbal/relational bullying scores, but these differences were not significant ($t(141) = 0.987, p = 0.676$). Physical bullying scores were higher in White adolescents than in Black adolescents, and these differences were also non-significant ($t(141) = 0.482, p = 0.489$). Cyberbullying scores were higher in Black adolescents than in White adolescents ($t(139) = -0.863, p = 0.390$); but again, these differences were non-significant.

White adolescents had higher CESD-10 scores than Black/other adolescents, but these differences were non-significant ($t(141) = 1.10, p = 0.273$). These same results held true for the cortisol diurnal rhythm with slightly larger change from AM to PM for the Black/other adolescents as compared to the White
adolescents; but these differences were non-significant ($t (141) = 0.346, p = 0.730$).

Socioeconomic status

With regard to SES, the SLE scores were higher for adolescents who received free or reduced-cost lunch as compared to those with full pay or other lunch status, though not significantly ($t (141) = 0.944, p = 0.347$). LCU scores were also higher for adolescents who received free/reduced-cost lunch status as compared to full pay and other lunch status, though not significantly ($t (141) = 1.412, p = 0.160$). PSS-10 scores were higher, though not significantly ($t (141) = 0.993, p = 0.322$) for adolescents who received free/reduced-cost lunch category as compared to adolescents who received full pay lunch or brought lunch from home. PECK scores were also higher in the free/reduced-cost lunch category as compared to the full pay/other lunch category; however, these differences in scores were non-significant ($t (141) = 1.201, p = 0.232$).

Adolescents who received free or reduced-cost lunch had higher, though not significantly different, verbal/relational bullying scores ($t (141) = 1.347, p = 0.180$). Physical bullying scores ($t (141) = 0.595, p = 0.553$) and cyberbullying scores ($t (139) = 0.979, p = 0.329$) were higher for those who received free or reduced-cost lunch as compared to adolescents who were full pay/other. Cultural bullying scores were only slightly higher for the free/reduced-lunch category, but these were non-significant differences ($t (141) = 0.563, p = 0.574$).

CESD-10 scores were slightly higher for free reduced-cost lunch adolescents as compared to the full price lunch status or those who brought lunch
from home; however, these differences were not significant ($t(141) = 0.679, p = 0.795$). The full pay/other lunch category had a slightly larger drop in the cortisol diurnal rhythm, although this difference was non-significant ($t(141) = 0.892, p = 0.374$) when comparing the free/reduced-cost lunch to the full price lunch or those who brought lunch from home.

**Gender identity**

Adolescents who self-reported as homosexual/bisexual/other had higher scores in SLE ($t(141) = -1.9, p = 0.059$); however, these differences were not significant as compared to heterosexual adolescents. There were significant differences in LCU scores ($t(141) = -2.530, p = 0.013$), as well as PSS-10 ($t(141) = -3.972, p < 0.001$), and PECK scores in adolescents who self-reported as homosexual/bisexual/other as compared to heterosexual adolescents ($t(141) = -3.039, p = 0.003$).

When examining subscales of bullying, adolescents who self-reported as homosexuals/bisexuals/unsure had significantly higher verbal/relational bullying scores as compared to heterosexuals ($t(141) = -3.95, p < 0.001$). Adolescents who self-reported as homosexual/bisexual/unsure had higher scores, though not significant in cyberbullying ($t(139) = -1.842, p = 0.068$), physical bullying ($t(139) = -0.233, p = 0.816$), or cultural bullying ($t(141) = -1.218, p = 0.225$) as compared those who self-reported as heterosexuals.

There were significant gender identity differences in the depressive symptom scores, equal variances not assumed ($t(141) = -4.191 p < 0.001$) with homosexual/bisexual/unsure adolescents scoring higher on the CESD-10 than
heterosexual adolescents. There was a larger drop in the cortisol diurnal rhythm in
the homosexual/bisexual/unsure adolescents as compared to those adolescents
who reported being heterosexual, but these differences were again non-significant
\( t (141) = 0.839, p = 0.403. \)

*Pubertal status*

Adolescents who self-reported late to post-pubertal status had significantly
higher SLE scores, equal variances not assumed \( t (136.79) = -2.873, p = 0.005 \),
LCU scores, equal variances not assumed \( t (141) = -2.556, p = 0.026 \), and PSS-
10 scores \( t (132) = -2.523, p = 0.013 \). PECK scores were higher in adolescents
who self-reported as late to post-pubertal status as compared to adolescents who
self-reported as early to mid-pubertal status, although these differences were not
significant \( t (141) = 0.153, p = 0.878 \).

Adolescents who self-reported as late to post pubertal status had higher
scores, though not significantly different in verbal/relational bullying \( t (141) = -0.368, p = 0.713 \) and cyberbullying \( t (139) = -0.567, p = 0.571 \) as compared to
early to mid-pubertal status. Physical bullying scores were slightly higher for the
early to mid-pubertal status as compared to the late to post-pubertal status,
although these differences were also non-significant, equal variances not assumed
\( t (86.235) = 1.577, p = 0.118 \). Cultural bullying scores were slightly lower,
though differences were not significant, for those in late to post pubertal status as
compared to those who were early to mid-pubertal status \( t (141) = 1.300, p =
0.196 \).
Adolescents who self-reported late to post-pubertal status had a significantly wider range of change from AM to PM cortisol ($t(141) = 2.592, p = 0.011$) as compared to adolescents who self-reported as early to mid-pubertal status. There were higher scores, though not significant, on the CESD-10 in the late to post-pubertal adolescents as compared to the early to mid-pubertal status adolescents ($t(132) = -0.688, p = 0.492$).

Table 15

*Independent Samples t-test: Means and SD of Independent Variables by Covariates (N = 143)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>SLE</th>
<th>LCU</th>
<th>PSS-10</th>
<th>PECK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13(7.9)</td>
<td>378(297)</td>
<td>17(7.6)</td>
<td>18(20)</td>
</tr>
<tr>
<td>Female</td>
<td>14(11.5)</td>
<td>420(305)</td>
<td>20(6.9)*</td>
<td>14(12)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>15(11)</td>
<td>416(319)</td>
<td>19(7.5)</td>
<td>15(14)</td>
</tr>
<tr>
<td>Black/other</td>
<td>12(7.6)</td>
<td>374(240)</td>
<td>19(7)</td>
<td>16(18)</td>
</tr>
<tr>
<td><strong>SES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free/reduced</td>
<td>15(9.2)</td>
<td>443(282)</td>
<td>20(6.8)</td>
<td>17(16.6)</td>
</tr>
<tr>
<td>Full pay/other</td>
<td>13(11.4)</td>
<td>372(317)</td>
<td>18(7.7)</td>
<td>14(13.7)</td>
</tr>
<tr>
<td><strong>Gender ID</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>13(10)</td>
<td>379(294)</td>
<td>18(6.8)</td>
<td>14(14.2)</td>
</tr>
<tr>
<td>Homosexual/other</td>
<td>18(9.7)</td>
<td>557(309)</td>
<td>24(7.5)*</td>
<td>24(17.3)</td>
</tr>
<tr>
<td><strong>Pubertal status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early-mid</td>
<td>11(6.7)</td>
<td>326(222)</td>
<td>17(8)</td>
<td>15(18)</td>
</tr>
<tr>
<td>Late-post</td>
<td>16(11.6)</td>
<td>445(328)*</td>
<td>20(6.7)*</td>
<td>15(13.5)</td>
</tr>
</tbody>
</table>

*Note.* *p < 0.05 level*
Table 16

*Independent Samples t-test: Mean (SD) - Types of Bullying by Covariates-N = 143*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Verbal/rel.</th>
<th>Cyber</th>
<th>Physical</th>
<th>Cultural</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
<td>$\bar{x}$ (SD)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8.3(9.2)</td>
<td>2.0(1.7)</td>
<td>4.4(5.3)*</td>
<td>1.7(2.8)</td>
</tr>
<tr>
<td>Female</td>
<td>7.9(7.1)</td>
<td>1.7(2.7)</td>
<td>1.4(2.5)</td>
<td>0.8(1.7)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8.2(7.7)</td>
<td>1.7(2.6)</td>
<td>2.5(4.1)</td>
<td>0.8(1.7)</td>
</tr>
<tr>
<td>Black/other</td>
<td>7.5(8.3)</td>
<td>2.2(5.2)</td>
<td>2.3(5.3)</td>
<td>2.0(3.4)*</td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free/reduced</td>
<td>8.9(8.5)</td>
<td>2.1(1.5)</td>
<td>2.6(4.0)</td>
<td>1.2(2.5)</td>
</tr>
<tr>
<td>Full pay/other</td>
<td>7.1(7.2)</td>
<td>2.2(4.0)</td>
<td>2.2(4.0)</td>
<td>1.0(1.8)</td>
</tr>
<tr>
<td>Gender ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>7.0(10)</td>
<td>1.6(3.3)</td>
<td>2.4(4.1)</td>
<td>1.0(2.1)</td>
</tr>
<tr>
<td>Homosexual/other</td>
<td>14(10)*</td>
<td>3.2(3.9)</td>
<td>2.6(3.4)</td>
<td>1.6(2.8)</td>
</tr>
<tr>
<td>Pubertal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early-mid</td>
<td>7.7(8.3)</td>
<td>1.6(4.5)</td>
<td>3.2(4.1)</td>
<td>1.4(3.2)</td>
</tr>
<tr>
<td>Late-post</td>
<td>8.2(7.7)</td>
<td>2.0(2.7)</td>
<td>2.1(0.4)</td>
<td>1.0(0.2)</td>
</tr>
</tbody>
</table>

*Note.* *p < 0.001
Table 17

*Independent Samples t-test: Dependent and Cortisol Diurnal Rhythm by Covariates, N = 143*

<table>
<thead>
<tr>
<th>Variable</th>
<th>CESD-10 $\bar{x}$ (SD)</th>
<th>Cortisol diurnal rhythm $\bar{x}$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8.7 (6.6)</td>
<td>-0.04 (0.09)</td>
</tr>
<tr>
<td>Female</td>
<td>9.1 (6.3)</td>
<td>-0.09 (0.16)*</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>9.3 (6.4)</td>
<td>-0.07 (0.14)</td>
</tr>
<tr>
<td>Black/other</td>
<td>7.9 (6.4)</td>
<td>-0.08 (0.17)</td>
</tr>
<tr>
<td><strong>SES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free/reduced</td>
<td>9.4 (6.3)</td>
<td>-0.06 (0.12)</td>
</tr>
<tr>
<td>Full pay/other</td>
<td>8.6 (6.4)</td>
<td>-0.08 (0.17)</td>
</tr>
<tr>
<td><strong>Gender ID</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>7.9 (5.5)</td>
<td>-0.07 (0.14)</td>
</tr>
<tr>
<td>Homosexual/other</td>
<td>15 (7.5)*</td>
<td>-0.11 (0.17)</td>
</tr>
<tr>
<td><strong>Pubertal status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early-mid</td>
<td>8.4 (7.2)</td>
<td>-0.03 (0.11)</td>
</tr>
<tr>
<td>Late-post</td>
<td>9.2 (5.9)</td>
<td>-0.09 (0.15)*</td>
</tr>
</tbody>
</table>

Note. *p < 0.05 level
Research Question 2

Are there correlations between stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms in 9th graders?

Hypothesis. There is a significant positive correlation between each of the variables (stressful life events, perceived stress, bullying, cortisol diurnal rhythm, and depressive symptoms).

Spearman’s Rho correlational analysis was used to explore for relationships between the variables. The above hypothesis was partially supported in that there were significant positive correlations with SLE, LCU, PSS-10, and PECK with CESD-10; however, there was not a significant correlation between cortisol diurnal rhythm and CESD-10. Relationships among the individual variables are discussed below with statistical findings summarized on Tables 18 and 19. Analysis for the research question and hypothesis is provided in narrative format below. This discussion includes the magnitude of the relationship and the effect size using Cohen’s standard (small magnitude = 0.10 – 0.20, medium magnitude = 0.30–0.49, large magnitude = > 0.50).

Stressful life events. Significant positive correlations were found between SLE and LCU (large magnitude, \( p < 0.01 \)), SLE and PSS-10 (medium magnitude, \( p < 0.01 \)), SLE and PECK (medium magnitude, \( p < 0.01 \)), SLE and CESD-10 (medium magnitude, \( p < 0.01 \)), SLE and cyberbullying (medium magnitude, \( p < 0.01 \)), SLE and physical bullying (small magnitude \( p < 0.05 \)). Stressful life events were positively correlated, though not significantly with cultural bullying, and cortisol diurnal rhythm.
Life Change Units. Life Change Units (LCU) had significant positive correlations with SLE (large magnitude, \( p < 0.01 \)), PSS-10 (medium magnitude, \( p < 0.01 \)), PECK (medium magnitude, \( p < 0.01 \)), and CESD-10 (medium magnitude, \( p < 0.01 \)). Significant positive correlations were found with the PECK subscales, between LCU and verbal/relational bullying (medium magnitude, \( p < 0.01 \)), cyberbullying (medium magnitude, \( p < 0.01 \)), and physical bullying (small magnitude, \( p < 0.01 \)). There was a non-significant positive correlation between LCU and cortisol diurnal rhythm, and cultural bullying.

Perceived Stress. Perceived stress had a significant positive correlation with CESD-10 (large magnitude, \( p < 0.01 \)), PECK scores (large magnitude, \( p < 0.01 \)), SLE (medium magnitude, \( p < 0.01 \)), and LCU (medium magnitude, \( p < 0.01 \)). Significant positive correlations were found between PECK subscales, between PSS-10 and verbal/relational bullying (large magnitude, \( p < 0.01 \)), physical bullying (medium magnitude, \( p < 0.01 \)), cyberbullying (small magnitude), and cultural bullying (small magnitude, \( p < 0.01 \)). Perceived stress was negatively correlated, though not significantly with cortisol diurnal rhythm.

Personal Experiences Checklist. Significant positive correlations with PECK scores were found between depressive symptoms (large magnitude \( p < 0.01 \)), PSS-10 (large magnitude, \( p < 0.01 \)), SLE (medium magnitude, \( p < 0.01 \)), and LCU (medium magnitude, \( p < 0.01 \)). Cortisol diurnal rhythm was positively correlated, though not significantly with PECK.
Cortisol Diurnal Rhythm

SLE, LCU, PECK, verbal bullying, cultural bullying, and physical bullying were found to be positively correlated with cortisol diurnal rhythm, though not significantly. A negative correlation was found with cortisol diurnal rhythm, PSS-10, and cyberbullying; although these correlations were not significant.

Table 18

Spearman’s Rho Bivariate Correlation Matrix: CESD-10, Cortisol Diurnal, SLE, LCU, PSS-10, and PECK (N = 143)

<table>
<thead>
<tr>
<th>Variable</th>
<th>CESD-10</th>
<th>Cortisol diurnal rhythm</th>
<th>SLE</th>
<th>LCU</th>
<th>PSS-10</th>
<th>PECK</th>
</tr>
</thead>
<tbody>
<tr>
<td>CESD-10</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol diurnal rhythm</td>
<td>-0.062</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>0.35*</td>
<td>0.001</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCU</td>
<td>0.42*</td>
<td>0.002</td>
<td>0.88*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS-10</td>
<td>0.73*</td>
<td>-0.044</td>
<td>0.34*</td>
<td>0.42*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PECK</td>
<td>0.65*</td>
<td>0.010</td>
<td>0.37*</td>
<td>0.40*</td>
<td>0.59*</td>
<td>1</td>
</tr>
</tbody>
</table>

Note. * p < 0.01
Table 19

Bivariate Correlation Matrix: CESD-10, Cortisol Diurnal Rhythm, and 4 Types of Bullying

<table>
<thead>
<tr>
<th>Variable</th>
<th>CESD-10</th>
<th>Cortisol diurnal rhythm</th>
<th>Verbal/Relational bullying</th>
<th>Cyber bullying</th>
<th>Cultural Bullying</th>
<th>Physical Bullying</th>
</tr>
</thead>
<tbody>
<tr>
<td>CESD-10</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol diurnal rhythm</td>
<td>-0.062</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal/Relational bullying</td>
<td>0.61*</td>
<td>0.034</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyber bullying</td>
<td>0.35*</td>
<td>-0.007</td>
<td>0.48*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultural bullying</td>
<td>0.31*</td>
<td>0.150</td>
<td>0.42*</td>
<td>0.27*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Physical bullying</td>
<td>0.49*</td>
<td>0.040</td>
<td>0.61*</td>
<td>0.42*</td>
<td>0.42*</td>
<td>1</td>
</tr>
</tbody>
</table>

Note. * p < 0.01

Research Question 3

Do stressful life events, perceived stress, bullying, and cortisol diurnal rhythm influence depressive symptoms in 9th grade adolescents after controlling for covariates of gender, race, socioeconomic status (SES), gender identity, and pubertal status?

Hypothesis. Each independent variable (stressful life events, perceived stress, cortisol diurnal rhythm, and bullying) will separately account for a percentage of the variance in depressive symptoms. Cumulatively, there will be a significant percentage of the variance in depressive symptoms explained by the combination of the three
independent variables after controlling for gender, race, gender identity, socioeconomic status (SES), and pubertal status.

General linear models were computed examining stressful life events, perceived stress, bullying, and cortisol diurnal rhythm influence on depressive symptoms in 9th graders. The assumptions of linearity, normally distributed errors, and uncorrelated errors were checked. Because the cortisol diurnal rhythm variable was not normally distributed, standardized cortisol diurnal rhythm values were used in this analysis. Hypothesis three was partially supported. Analysis for the research question and hypothesis is provided in narrative format below. Descriptive statistics for these variables are presented on Table 14. The models (Models 1-4) are summarized on Tables 20 and 21.

Model 1 included the covariates: gender, race, SES, gender identity and pubertal status. Model 2 included the independent variables: SLE, LCU, PSS-10, and PECK, and Model 3 included the cortisol diurnal rhythm (change from AM to PM). Model 4 included: covariates, independent variables and the diurnal cortisol rhythm all in the same model to observe for change in the $R^2$, in order to determine which model has the highest proportion of the variance with regard to depressive symptoms. Covariates of race, SES, gender identity, and pubertal status were re-coded into two categories for appropriate analysis.

Eta squared is a measure of effect size indicating the proportion of the variance in the dependent variable explained by the independent variable (Grimm, & Yarnold, 2000, Polit & Beck, 2012). When interpreting Eta squared ($\eta^2$), the
effect sizes used in this study were as follows: 0.02 = small, 0.13 = medium and 0.26 = large effect size (Cohen, 1988).

When examining Model 1 (Table 20 & 21), the covariates together accounted for 17.9% of the variance in depressive symptoms. However, the only significant variable was gender identity ($\beta = -7.275, \text{SE} = 1.446, p < 0.001$, $\text{Partial } \eta^2 = 0.160$), a medium effect size. When examining Model 2 (Table 20), the independent variables together accounted for 62.7% of the variance in depressive symptoms ($R^2 = 0.627$, $\text{Adj. } R^2 = 0.616, p < 0.001$). However the only two significant contributors to this model were PSS-10 ($\beta = 0.478, p < 0.001$, $\text{Partial } \eta^2 = 0.368$), large effect size, and PECK ($\beta = 0.142, p < 0.001$, $\text{Partial } \eta^2 = 0.178$) with a medium effect size.

In Model 3 (Tables 20 & 21), cortisol diurnal rhythm had no relationship with depressive symptoms ($R^2 = 0.000$, $\text{Adjusted } R^2 = -0.007$, $F (1,138) = 0.000, p = 0.882$), ($B = 0.004, SE = 0.013, t = 0.276, p = 0.789$). When examining Model 4 (Table 20 & 21), the covariates, independent variables, and cortisol diurnal rhythm all accounted for 67% of the variance in depressive symptoms ($R^2 = 0.670$, $\text{Adj. } R^2 = 0.644, p < 0.001$). The variables that contributed the most to depressive symptoms were PSS-10 ($F (1,138) = 72.53, p < 0.001$, $\text{Partial } \eta^2 = 0.362$), ($\beta = 0.480, SE = 0.056, t = 8.517, p < 0.001$) with a large effect size, and PECK ($F (1,138) = 20.592, p < 0.001$, $\text{Partial } \eta^2 = 0.139$) with a medium effect size, ($\beta = 0.122, SE = 0.027, t = 4.538, p < 0.001$), and gender identity ($F (1,138) = 12.275, p < 0.001$, $\text{Partial } \eta^2 = 0.088$), ($\beta = -3.454, SE = 0.986, t = -3.504, p < 0.001$) with a small effect size.
Table 20

*General Linear Models 1, 2, 3, 4*

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>Adj $R^2$</th>
<th>df</th>
<th>$F$</th>
<th>p-value</th>
<th>$P \text{ Eta}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>0.179</td>
<td>0.149</td>
<td>5</td>
<td>5.815</td>
<td>0.000*</td>
<td>0.179</td>
</tr>
<tr>
<td>Gender</td>
<td>1</td>
<td>1.137</td>
<td>286</td>
<td>5.815</td>
<td>0.000*</td>
<td>0.179</td>
</tr>
<tr>
<td>Race</td>
<td>1</td>
<td>1.198</td>
<td>286</td>
<td>25.296</td>
<td>0.000*</td>
<td>0.160</td>
</tr>
<tr>
<td>SES</td>
<td>1</td>
<td>1.313</td>
<td>286</td>
<td>0.195</td>
<td>0.659</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender identity</td>
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<td>25.296</td>
<td>286</td>
<td>0.195</td>
<td>0.659</td>
<td>0.001</td>
</tr>
<tr>
<td>Pubertal status</td>
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<td>286</td>
<td>0.195</td>
<td>0.659</td>
<td>0.001</td>
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<th>p-value</th>
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<tr>
<td>LCU score</td>
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<td>0.178</td>
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<tr>
<td>PSS-10 score</td>
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<td>56273</td>
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<th>$F$</th>
<th>p-value</th>
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<td>1</td>
<td>0.076</td>
<td>0.783</td>
<td>0.001</td>
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</tr>
<tr>
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<td>0.783</td>
<td>0.001</td>
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<th>$F$</th>
<th>p-value</th>
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<td>0.644</td>
<td>10</td>
<td>25.945</td>
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<td>0.670</td>
<td></td>
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<tr>
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<td>25945</td>
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<td>0.021</td>
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<td>0.015</td>
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<td>0.006</td>
<td>25945</td>
<td>0.000*</td>
<td>0.000</td>
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<tr>
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<td>25945</td>
<td>0.000*</td>
<td>0.088</td>
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<td>0.139</td>
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*Note. * $p < 0.05$
Table 21

Beta coefficients, Standard errors, t- values and p-values for Models 1, 2, 3, 4

<table>
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<th>Model 1</th>
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<tbody>
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</tr>
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<td>1.078</td>
<td>1.066</td>
<td>0.288</td>
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<tr>
<td>Gender - Female</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>1.310</td>
<td>1.197</td>
<td>1.095</td>
<td>0.276</td>
</tr>
<tr>
<td>Race-Black/Other</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SES- Free-reduced</td>
<td>1.179</td>
<td>1.029</td>
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<td>0.254</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
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Model 2

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<th>p</th>
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</thead>
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<td>-2.564</td>
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<tr>
<td>SLE</td>
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<td>0.063</td>
<td>0.637</td>
<td>0.536</td>
</tr>
<tr>
<td>LCU</td>
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<td>0.002</td>
<td>-0.343</td>
<td>0.732</td>
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<tr>
<td>PSS-10</td>
<td>0.478</td>
<td>0.054</td>
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</tr>
<tr>
<td>PECK</td>
<td>0.142</td>
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<td>5.380</td>
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</table>

Model 3

<table>
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<th>p</th>
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</thead>
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<tr>
<td>Intercept</td>
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<td>0.145</td>
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<td>0.013</td>
<td>0.276</td>
<td>0.783</td>
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</table>

Model 4

<table>
<thead>
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<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>Intercept</td>
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<td>2.139</td>
<td>0.400</td>
<td>0.690</td>
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<tr>
<td>Gender- Male</td>
<td>1.248</td>
<td>0.784</td>
<td>1.670</td>
<td>0.097</td>
</tr>
<tr>
<td>Gender - Female</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Race-White</td>
<td>1.092</td>
<td>0.781</td>
<td>1.398</td>
<td>0.164</td>
</tr>
<tr>
<td>Race- Black/other</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SES-Free/Reduced</td>
<td>0.053</td>
<td>0.677</td>
<td>0.078</td>
<td>0.938</td>
</tr>
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<td>SES-Full Price</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender identity- Hetero</td>
<td>-3.454</td>
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<td>0.001*</td>
</tr>
<tr>
<td>Homosexual/bisexual/unsure</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pubertal status</td>
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<td>0.062</td>
<td>0.786</td>
<td>0.434</td>
</tr>
<tr>
<td>LCU</td>
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<td>0.002</td>
<td>-0.654</td>
<td>0.514</td>
</tr>
<tr>
<td>PSS-10</td>
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<td>8.517</td>
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<td>4.538</td>
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<tr>
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<td>-0.004</td>
<td>0.334</td>
<td>-0.013</td>
<td>0.990</td>
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</table>

Note. * p < 0.05. 0a - parameter is set to zero
Research Question 4

Does cortisol diurnal rhythm mediate the relationship between stressful life events and depressive symptoms after controlling for age, gender, race, socioeconomic status (SES), gender identity, and pubertal status?

Hypothesis. Cortisol diurnal rhythm mediates the relationship between bullying and depressive symptoms after controlling for gender, race, socioeconomic status, gender identity, and pubertal status.

Using Baron and Kenny’s Mediation Model steps, the following statistical steps were taken. A general linear model for Step 1 was used to determine whether the predictor variable (X) is correlated with the outcome variable (Y) (depressive symptoms), showing that there is an effect that may be mediated. The models used to address the research questions are shown in Table 21. The hypothesis was not supported, and the analysis for the research question and hypothesis is provided in the narrative below.

In Step 1, a General Linear Model 1 (Table 22 & 23) was developed with only the covariates to determine if this model was significant and if any of the covariates contributed to depressive symptoms (CESD-10). Model 1 was significant with regard to the variance in depressive symptoms ($R^2 = 0.186, Adj. R^2 = 0.156, p < .001$); however, gender identity was the only significant variable in Model 1($F (1,) = 26.392, p < 0.001$). In the next, Model 2 (Tables 22 & 23), the stressful life event (SLE) variable was added and was again significant ($R^2 = 0.221, Adj. R^2 = 0.187, p < 0.001$), with SLE contributing significantly to the variance in depressive symptoms), ($F (1, 6) = 6.120, p = 0.015$), and
gender identity contributing more significantly toward the variance of depressive symptoms \((F (1, 6) = 23.248, p < 0.001)\).

In Step 2, the investigator must determine whether the \((X)\) is correlated with the mediating variable \((M)\) (cortisol), the mediating variable \((M)\) is seen as the outcome variable \((Y)\) in the General Linear Model equation, and the predictor variable is seen as the \(X\). For Step 2, Model 3 was developed by including the covariates, the independent variable SLE, while the mediating variable (cortisol diurnal rhythm) became the dependent variable. A standardized cortisol diurnal rhythm value was used in this model. Based on analysis, Model 3 did not significantly explain the dependent variable \((R^2 = 0.001, Adj. R^2 = -0.007, p = 0.783)\). Model 3 provides the findings that covariates and the SLE did not provide a significant contribution \((p = 0.351)\) to explaining the variance in cortisol diurnal rhythm; therefore, the data analysis did not support cortisol diurnal rhythm as a mediator.

For Step 3, the investigator must determine whether the mediator \((M)\) affects the dependent variable \((Y)\); \((Y)\) again is the outcome variable in the General Linear Model equation, and the independent variable \((X)\) and the mediator variable \((M)\) are predictors. The mediator and the outcome variable must be correlated because they are both caused by the initial variable \((X)\).

For this step, Model 4 (Tables 22 & 23) was developed, which included the covariates, the independent variable, SLE, and the mediating variable, cortisol diurnal with the dependent variable being depressive symptoms (CESD-10). Results showed that the model was significant \((R^2 = 0.227, Adj. R^2 = 0.187, p < 0.001)\); however, the only significant contributors to depressive symptoms remained Gender identity \((F (1, 7)\)
= 23.739, \( p < 0.001 \)) and SLE \( (F (1, 7) = 6.496, \ p = 0.012) \). Cortisol diurnal rhythm did
not contribute significantly towards the variance in depressive symptoms \( (F (1, 7) = 1.054, \ p = 0.307) \). At this point, the mediation analysis was stopped because cortisol
diurnal rhythm was not related to the SLE, or the CESD-10 scores. Research questions
4, 5, and 6 were not supported. Research questions 5 and 6 were not examined due to the
lack of relationship of cortisol diurnal rhythm to depressive symptoms.
Table 22

*Mediation using General Linear Model -1, 2, 3, 4*

<table>
<thead>
<tr>
<th>Model 1</th>
<th>$R^2$</th>
<th>Adj $R^2$</th>
<th>df</th>
<th>$F$</th>
<th>sig</th>
<th>P Eta $^2$</th>
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<td></td>
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<td>0.156</td>
<td>5</td>
<td>6.269</td>
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<th>df</th>
<th>$F$</th>
<th>sig</th>
<th>P Eta $^2$</th>
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<table>
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<th>df</th>
<th>$F$</th>
<th>sig</th>
<th>P Eta $^2$</th>
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</table>

Note. Cortisol diurnal rhythm is the Dependent variable

<table>
<thead>
<tr>
<th>Model 4</th>
<th>$R^2$</th>
<th>Adj $R^2$</th>
<th>df</th>
<th>$F$</th>
<th>sig</th>
<th>P Eta $^2$</th>
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<td></td>
</tr>
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<td>0.307</td>
<td>0.008</td>
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<td></td>
</tr>
<tr>
<td>SLE</td>
<td>1</td>
<td>6.496</td>
<td>0.012*</td>
<td>0.046</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *p < 0.05, Depressive symptoms - DV
Table 23

**Beta coefficients, Standard errors, t-values and p-values for Models 1, 2, 3, 4**

<table>
<thead>
<tr>
<th>Model 1</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>10.380</td>
<td>2.772</td>
<td>3.744</td>
<td>0.000*</td>
</tr>
<tr>
<td>Gender - Male</td>
<td>1.088</td>
<td>1.096</td>
<td>0.993</td>
<td>0.323</td>
</tr>
<tr>
<td>Gender - Female</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Race - White</td>
<td>1.714</td>
<td>1.216</td>
<td>1.410</td>
<td>0.161</td>
</tr>
<tr>
<td>Race - Black/Other</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SES - Free-reduced</td>
<td>1.179</td>
<td>1.029</td>
<td>0.937</td>
<td>0.350</td>
</tr>
<tr>
<td>SES – Full price</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender ID- hetero</td>
<td>-7.225</td>
<td>1.406</td>
<td>-5.137</td>
<td>0.000*</td>
</tr>
<tr>
<td>Homosexual/bisexual/unsure</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>0.281</td>
<td>0.220</td>
<td>1.277</td>
<td>-0.204</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>9.451</td>
<td>2.748</td>
<td>3.440</td>
<td>0.000*</td>
</tr>
<tr>
<td>Gender - Male</td>
<td>1.061</td>
<td>1.076</td>
<td>0.986</td>
<td>0.326</td>
</tr>
<tr>
<td>Gender - Female</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Race - White</td>
<td>1.377</td>
<td>1.202</td>
<td>1.146</td>
<td>0.254</td>
</tr>
<tr>
<td>Race - Black/other</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SES - Free-reduced</td>
<td>0.696</td>
<td>1.030</td>
<td>0.676</td>
<td>0.500</td>
</tr>
<tr>
<td>SES – Full price</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender ID- Hetero</td>
<td>-6.728</td>
<td>1.385</td>
<td>-4.822</td>
<td>0.000*</td>
</tr>
<tr>
<td>Homosexual/bisexual/unsure</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>0.199</td>
<td>0.218</td>
<td>0.910</td>
<td>0.365</td>
</tr>
<tr>
<td>SLE</td>
<td>0.119</td>
<td>0.048</td>
<td>2.474</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 3</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.287</td>
<td>0.469</td>
<td>0.613</td>
<td>0.541</td>
</tr>
<tr>
<td>Gender - Male</td>
<td>0.235</td>
<td>0.184</td>
<td>1.280</td>
<td>0.203</td>
</tr>
<tr>
<td>Gender-Female</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Race - White</td>
<td>0.227</td>
<td>0.205</td>
<td>1.108</td>
<td>0.270</td>
</tr>
<tr>
<td>Race – Black/Other</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SES – Free-reduced</td>
<td>0.219</td>
<td>0.176</td>
<td>1.244</td>
<td>0.216</td>
</tr>
<tr>
<td>SES – Full price</td>
<td>0a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pubertal status</td>
<td>-0.071</td>
<td>0.037</td>
<td>-1.918</td>
<td>0.057</td>
</tr>
<tr>
<td>SLE</td>
<td>-0.008</td>
<td>0.008</td>
<td>-0.937</td>
<td>0.351</td>
</tr>
</tbody>
</table>

*Note.* Cortisol diurnal rhythm, dependent variable
### Note

* $p < 0.05$ 0 - parameter is set to zero

### Additional Statistical Analysis

Adolescents were asked to respond to an additional question about their current living arrangements in the demographic portion of the online questionnaire. Potential responses included: 1) I live with my mom and dad, 2) I live with my mom, 3) I live with my dad, 4) I live with my grandparents, or 5) Other living arrangement. The majority of adolescents reported living with both parents (57.4%). A minority of adolescents ($n = 11$ or 7.7%) reported having other living arrangements including seven adolescents living with mom and stepdad, one living partially with mom and partially with grandparents, one with an aunt and uncle, one living with cousins, and one living with foster parents. Results summarized on Table 24.
Tests of association (ANOVA) were conducted to determine differences in the five living arrangement categories with regard to variables, SLE, LCU, PSS-10, PECK, and CESD-10. Adolescents who reported living with grandparents had the highest scores for independent variables (SLE, LCU, PSS-10, and PECK). However, only the PSS-10 score was significantly different in those who lived with grandparents as compared to those living with mom and dad, mom only, dad only, or other situations. Because there are only five adolescents (3.5%) in the category of living with grandparents, the results should be interpreted with caution. Results are summarized on Table 25.
Table 25

**ANOVA: Living arrangement with dependent and independent variables**

<table>
<thead>
<tr>
<th></th>
<th>SLE</th>
<th>LCU</th>
<th>PSS-10</th>
<th>PECK</th>
<th>CESD-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x̄ (SD)</td>
<td>x̄ (SD)</td>
<td>x̄ (SD)</td>
<td>x̄ (SD)</td>
<td>x̄ (SD)</td>
</tr>
<tr>
<td>Mom &amp; Dad</td>
<td>13.7(11.9)</td>
<td>376(320)</td>
<td>18(7)</td>
<td>15(17)</td>
<td>8.5(6)</td>
</tr>
<tr>
<td>Mom only</td>
<td>14.5(8.9)</td>
<td>419(286)</td>
<td>19(8)</td>
<td>14(12)</td>
<td>8.7(6)</td>
</tr>
<tr>
<td>Dad only</td>
<td>11.6(5.8)</td>
<td>391(216)</td>
<td>19(6)</td>
<td>12(13)</td>
<td>8.1(6)</td>
</tr>
<tr>
<td>Grandparents</td>
<td>18.2(10.3)</td>
<td>583(353)</td>
<td>27(7)</td>
<td>23(19)</td>
<td>16(9)</td>
</tr>
<tr>
<td>Other</td>
<td>15.91(5.5)</td>
<td>515(228)</td>
<td>21(7)</td>
<td>19(13)</td>
<td>11(7)</td>
</tr>
</tbody>
</table>

*Note.* *p* < 0.05

Adolescents were asked three questions at the end of the study to determine if any of the questions asked were upsetting and which of the questions upset them. They were also asked if they would be interested in participating in a future study similar to the current study (Table 26). Of the six adolescents who responded that something was upsetting to them, two adolescents said that providing personal information was upsetting. One adolescent said that the questions about stress were upsetting, and three adolescents said that the questions about depressive symptoms were upsetting (Table 26).
Table 26

*Frequency data: Was anything upsetting about the survey? Interested in a future study?*

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Was anything upsetting?</td>
<td>6(4.2)</td>
<td>137(95.8)</td>
</tr>
<tr>
<td>Interested in a future study?</td>
<td>122(85.9)</td>
<td>20(14.1)</td>
</tr>
</tbody>
</table>

Data were collected as to the time from the beginning of the cortisol AM saliva collection and the cortisol PM saliva collection. Length of time between AM and PM collection periods were examined in comparison to the three groups of cortisol diurnal rhythm (normal cortisol diurnal rhythm, flat or blunted cortisol diurnal rhythm, and opposite cortisol diurnal rhythm); therefore, significant differences were discovered. Adolescents who had normal cortisol diurnal rhythm had the longest length of time between AM and PM saliva collection (mean = 4 hrs, 55 mins. SD = 1 hr, 05 mins.), while adolescents who had opposite diurnal rhythm pattern had the shortest time period between the AM and PM data collection (mean = 4 hrs, 08 mins. SD = 1 hr, 12 mins.) Results are summarized on Table 27.
Table 27

ANOVA: Time from Cortisol AM and PM saliva collection compared to Normal cortisol diurnal rhythm, Flat/blunted cortisol diurnal rhythm, and Opposite cortisol diurnal rhythm (N = 143)

<table>
<thead>
<tr>
<th>Category</th>
<th>x̄ # of hours(SD)</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4:55 (1:05)*</td>
<td>6.583</td>
<td>0.002</td>
</tr>
<tr>
<td>Flat/blunted</td>
<td>4:23 (1:04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opposite</td>
<td>4:08 (1:12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *p < 0.05

Correlations of Covariates with SLE, LCU, PSS-10, PECK, and Cortisol Diurnal Rhythm

Correlations were examined among each of the covariates with the independent variables (SLE, LCU, PSS-10, PECK), the dependent variable (CESD-10), and cortisol diurnal rhythm. This discussion includes the magnitude of the relationship and the effect size using Cohen’s standard (small magnitude = 0.10-0.20, medium magnitude = 0.30 – 0.49, large magnitude > 0.50).

Gender. Gender had a significant positive correlation with PSS-10 (small magnitude), Gender identity, (small magnitude), and pubertal status (small magnitude). There were significant negative correlations between gender and cultural bullying (small magnitude), physical bullying (small magnitude), and cortisol diurnal rhythm (small magnitude). Other positive yet non-significant correlations with gender were SLE, LCU, verbal bullying, cyberbullying, depressive symptoms. PECK and race were negatively correlated with gender; yet these correlations were non-significant.
Race. There was a significant negative correlation between race and SES (small magnitude) and a significant positive correlation with cultural bullying (small magnitude). Other non-significant negative correlations with race were SLE, LCU, PSS-10, verbal/relational bullying, cyberbullying, physical bullying, depressive symptoms, and gender. Race had a positive, yet non-significant correlation with PECK and cortisol diurnal rhythm.

Socioeconomic status. School lunch status (SES) had a significant negative correlation with LCU (small magnitude) and race (small magnitude). Other variables that were negatively correlated with SES were SLE, PSS-10, PECK, verbal/relational bullying, cyberbullying, physical bullying, depressive symptoms, cortisol diurnal rhythm, gender identity, and pubertal status; however, all of these negative correlations were non-significant. There were two positive correlations seen between SES and cultural bullying, and SES and gender; however these correlations were non-significant.

Gender identity. Gender identity had significant positive correlations with SLE (small magnitude), LCU (small magnitude), PSS-10 (small magnitude), PECK (small magnitude), CESD-10 (small magnitude), verbal relational bullying (small magnitude), and cyberbullying (small magnitude), and gender (small magnitude). Gender identity had non-significant negative correlations with race and SES. Gender identity had non-significant positive correlations with cultural bullying, physical bullying, and pubertal status.

Pubertal Status. Pubertal status had a positive significant correlation with SLE (small magnitude), LCU (small magnitude), PSS-10 (small magnitude),
cyberbullying (small magnitude), CESD-10 (small magnitude), and gender (small magnitude). Pubertal status had a significant negative correlation with cortisol diurnal rhythm and a non-significant negative correlation with race.

Depressive Symptoms Scores and Bullying scores by categories

In the current study, higher bullying scores were associated with higher depressive symptom scores. Categories were developed by the investigator to determine the breakdown of bullying scores in the sample. Cross tabulations and Chi square tests were conducted to examine bullying and depressive symptoms scores by gender. Out of a total score of 128, three categories emerged (low, medium, and high scores). There were 30 male and 64 female adolescents who reported bullying scores of 0 to 15 (low scores), and 9 males and 22 females adolescents who reported bullying scores of 16 to 30 out of a total score of 128 (medium range scores). There were 10 males and 8 females who reported the highest bullying scores of 31 to 98 out of a total score of 128. A small number of adolescent males (10%) and females (21.9%) with very low bullying scores had clinically significant depressive symptoms. The majority of adolescent males (88.9%) and females (68.2%) who scored in the medium range of bullying scores had clinically significant depressive symptoms (CESD-10 > 9). The majority of the adolescent males (90%) and females (87.5%) who scored in the highest range of bullying scores also had clinically significant depressive symptoms. Pearson chi square testing was significant for males ($\chi^2 = 30.418, df = 2, p < 0.001$) and for females ($\chi^2 = 23.815, df = 2, p < 0.001$).
Summary

All variables and covariates collected were discussed in terms of descriptive analyses. Research questions were then discussed with regard to specific findings for individual questions and hypotheses. Additional analyses were run for data collected that were not included in the research questions but were interesting in relation to the overall study findings.

Research Question 1 included descriptive and bivariate analysis conducted for the independent variables (SLE, LCU, PSS-10, PECK, and types of bullying), cortisol diurnal rhythm, covariates (gender, race, gender identity, SES, and pubertal status) and the dependent variable (CESD-10). Adolescents reported an average of 14 SLE in the last year with 80% having above a normative LCU score of 170. The majority of adolescents (84%) scored from 11-30 out of a maximum score of 40 on the PSS-10. Females, adolescents who were late or post-pubertal, and those self-reporting as homosexual/bisexual or unsure of gender identity scored significantly higher on the PSS-10 as compared to males, adolescents who were early or mid-puberty, and those reporting as heterosexuals.

The majority of adolescents (64%) had a normal cortisol diurnal rhythm with smaller percentages (12.5%) having a flattened rhythm and 23% having an opposite diurnal rhythm. Bullying scores were low overall (20%); however the highest scores were in verbal/relational bullying. Males reported experiencing significantly more physical bullying, Blacks and other racial minorities reported more cultural bullying, and adolescents self-reporting homosexual/bisexual or unsure of gender identity had higher bullying scores overall. Clinically significant
depressive symptoms (CESD-10 > 9) were reported in 39.2% of adolescents with those adolescents self-reporting as homosexual/bisexual or unsure of gender identity reporting the highest bullying scores compared to other adolescents.

Research Question 2 revealed significant positive correlations ($p < 0.01$ level) for each of the independent variables (LCU, SLE, PSS-10, PECK, and CESD-10). The largest magnitude was the correlation between PSS-10 and CESD-10. The SLE, LCU, and PECK were small to medium magnitude for the correlation with CESD-10. There were also significant positive correlations ($p < 0.01$ level) among each type of bullying and depressive symptoms. The largest magnitude was between verbal/relational bullying. The other three types of bullying (cyber, physical, and cultural) had small to medium magnitude for the correlation with CESD-10. There were no significant correlations among cortisol AM, the cortisol PM, or cortisol diurnal rhythm and depressive symptoms. The hypothesis for research question #2 was partially supported.

A General Linear Model statistical analysis was used for Research Question 3. In model one (covariates only), gender identity was the only covariate that significantly ($p < 0.05$) contributed to depressive symptoms as a medium to large effect ($PEta^2 = 0.183$). In model 2 (independent variables only), perceived stress (PSS-10) and bullying (PECK) were the only independent variables that contributed significantly ($p < 0.05$) to depressive symptoms. PSS-10 had a large effect ($PEta^2 = 0.377$) and PECK had a medium effect ($PEta^2 = 0.169$). In model 3 (cortisol diurnal rhythm), there was no significant contribution towards depressive symptoms. In the final model 4 (covariates, independent variables, and
cortisol diurnal rhythm, there were three variables that contributed significantly to depressive symptoms and these were gender identity, perceived stress, and bullying. Perceived stress and bullying contributed 59.4% of the variance in depressive symptoms. Gender identity contributed only 0.9% towards the variance in depressive symptoms. The hypothesis for Research Question 3 was partially supported.

Research Question 4 was conducted using mediation analysis. After controlling for socioeconomic status, gender, race, gender id, and pubertal status, the independent variable (SLE) predicted a significant amount of the variance in depressive symptoms. However, there was no statistical relationship between cortisol diurnal rhythm (change from AM to PM) and depressive symptoms; therefore, this precluded the need for additional testing of cortisol diurnal rhythm for mediation. The statistical analysis for Research Questions 5 and 6 were not conducted because there was no statistical relationship between depressive symptoms and cortisol diurnal rhythm; therefore, this precluded the need for additional testing of cortisol diurnal rhythm for mediation.
CHAPTER FIVE

The primary goal of this study was to examine stressful life events, perceived stress, and bullying to determine the influence of these variables independently and cumulatively on depressive symptoms in a 9th grade adolescent population. In addition, cortisol (physiological biomarker of stress) was examined to determine if the cortisol diurnal rhythm has a mediating relationship with each of the independent variables (stressful life events, life change units, perceived stress, and bullying) and the dependent variable, depressive symptoms.

In this chapter a discussion of the study findings is presented. The major findings of the study are discussed along with findings related to the research questions and hypotheses. The conceptual framework will be discussed as well as study limitations and the implications for professional practice, policy, and future research.

Major Findings

A convenience study sample was recruited from two high schools located in a Southern U.S. state. The majority of the study sample participants were 14 year old, Caucasian, female, identifying themselves as heterosexuals, and approximately half of the adolescents received a free/reduced-cost lunch (proxy for socioeconomic status [SES]). The demographic characteristics of the two high schools were similar to the study sample. Adolescents in these schools for the current study were predominantly White (81%-85%) and fewer minority races (12%-18% Black, 2-3% other races) (D.
Collins and J. McCutchen, personal communication, June 5, 2014). When comparing the two cities in which the schools were located, the race is primarily White (63%-83%) with the next highest race category being Black (8%-12%). There were few American Indian, Asian, Hispanic and multi-racial individuals, but the percentage was no more than 2.3% in any of these categories.

Adolescents in this study reported high levels of stress (SLE, LCU, and PSS-10). On average, adolescents reported 14 stressful life events (SLE) occurring in the last year with 80% of adolescents having above the normative life change unit (LCU) score of 170, and the majority of adolescents (84%) having perceived stress scores (PSS-10) in the middle range (mean score = 11 to 30 out of a total score of 40). Significantly higher stress (SLE, LCU, and PSS-10) was reported by adolescents who identified themselves as late-to post-pubertal status and homosexual/bisexual or other minority sexual identity. The majority of adolescents (64%) had a normal cortisol diurnal rhythm with fewer (12.5%) having a flattened rhythm and 23% having an opposite diurnal rhythm. Females and those who self-reported late-to post-puberty also had a larger level of change between morning and afternoon cortisol (diurnal rhythm).

Adolescents’ reports of bullying (PECK scores) were low overall (20%) as compared to U.S. rates (26% to 28%) of adolescent bullying (Robers et al., 2012; Ryoo et al., 2014); however, adolescent reports of verbal/relational bullying were higher than physical, cyber, and cultural bullying. Male adolescents reported significantly more physical bullying than females. Black and other racial minority adolescents scored higher on cultural bullying scores as compared to White adolescents. Adolescents who self-reported being homosexual/bisexual/unsure had significantly higher bullying scores
overall, with particularly higher scores on verbal/relational bullying than heterosexuals. Clinically significant depressive symptoms (CESD-10 > 9) were self-reported in 39.2% of adolescents. Adolescents self-reporting as homosexual/bisexual or unsure had higher CESD-10 scores than heterosexuals.

Higher stress (SLE, LCU, PSS-10) and reports of bullying (PECK) were significantly associated with higher reports of depressive symptoms ($p = 0.01$). The highest magnitude of correlation was among perceived stress (PSS-10) and depressive symptoms (CESD-10); while the other variables had a small or medium magnitude correlation with depressive symptoms. The second hypothesis was partially supported.

In the predictive model, the independent variables of perceived stress (large effect size) and bullying (small effect size), and one covariate, gender identity (small effect size) contributed 67% ($p < 0.05$) of the variance in depressive symptoms. Perceived stress contributed the largest variance (48%) in depressive symptoms. The third hypothesis was partially supported. Effect sizes were: 0.02 = small, 0.13 = medium and 0.26 = large effect size.

In this study, the mediating hypotheses were not supported. While the independent variables (SLE, LCU, PSS-10, and PECK) were positively correlated with the dependent variable (CESD-10), there was no correlation between independent variables and cortisol diurnal rhythm, and no correlation between cortisol diurnal rhythm and the dependent variable (CESD-10).
Recruitment and Participation Rates

Response rates in this study were very good (55%). Blom-Hoffman et al., (2009) reported response rates for school based-studies ($n = 124$), finding response rates ranging from 11%-100% ($\bar{x} = 65.5\%$). Recruitment and participation success as seen in this study could be attributed to developing a strong relationship with school personnel who have influence over activities (e.g. nursing supervisor, teachers, and principal) well in advance of the study. Alibali and Nathan (2010) confirm that having a positive relationship with teachers and administrators in the school setting is a key to conducting a successful study. These researchers further stress that being patient, flexible, showing respect to teachers, students, staff, parents, and being considerate of guidelines provided by administrators are important issues to keep in mind when working in a school setting. Having a physical presence at the school, becoming familiar with the teachers, principal, librarian, computer teachers, and students, and having those in authoritative positions “buy into” the idea of conducting research in their school also contribute to the response rate (Unger et al., 2004).

One notable issue in a school setting is the importance of obtaining parental consent. Federal regulations mandate that active consent be provided for minors who participate in research that involves “minimal risk” (U.S. Department of Human Services, 2011). The current study included active parental consent. However, some studies are conducted in schools with only passive consent required, in these situations the participation rate is generally over 80% (McMorris et al., 2004).
Descriptive Findings of Health Variables (Pubertal Status and Smoking),
Cortisol (AM, PM), and Stressful Day

Pubertal status and smoking status were examined in this study, along with collecting saliva samples to measure morning (AM) and afternoon cortisol (PM) and a question about something stressful occurring on the day of data collection (Stressful Day). Findings related to these variables will be discussed, as they were not included in the original research questions.

*Pubertal Status*

Adolescents in this study self-reported pubertal status. The majority of males (73.3%) were mid-to late-pubertal status, and the majority of females (76.6%) were late to post-pubertal status. Although pubertal changes can vary by individual, these findings were in the range of expected pubertal development for adolescents’ aged 14 to 16 years (Patton & Viner, 2007). Tanner (1962) describes puberty as a time of adolescent growth spurt, not only in height, but in muscular, skeletal, and hormonal change. According to Walvoord (2010), the age of puberty has declined in females and males over the last 40 years.

Visual assessment of puberty status has been used in large national studies such as the National Health and Nutrition Examination Survey (NHANES), the Bogalusa Heart Study, and the Pediatric Research in the Office Setting (Walvoord, 2010). For females, breast development generally begins at around age 10.5 to 11.5 with menarche beginning at a mean age of 12.7. In males, the beginning stages of puberty generally begin between the ages of 10 to 12 with genital development and pubic hair reaching maturity between
the ages of 15 to 16 (Walvoord, 2010). Adolescents in this current study had similar pubertal status to those of the national studies discussed by Walvoord (2010).

Smoking Status

In the current study, a question about smoking (yes/no) was asked of adolescents. There were only 4.2% of the adolescents who were self-reported smokers. Adolescents’ smoking status self-report in this study were lower than smoking rates for Alabama adolescents (12%) (CDC, 2010) and lower than U. S. rates of adolescent smoking (9.2%) (CDC, 2015). The results of the current study should be viewed with caution in that there were small numbers of smokers. Smoking in the teen years is sometimes intermittent and can be reported inconsistently (Kaestle, 2015; Rubinstein, Rait, Sen & Shiffman, 2014).

Higher smoking rates (26%) and higher perceived stress was reported by Siqueira et al. (2000) in a study of adolescents’ aged 12 to 21 years. In the current study, while the cell sizes were small, the majority of adolescents who reported smoking self-reported their gender identity as homosexual/bisexual as compared to heterosexual. In a larger study, Azagba, Asbridge, Langille, and Baskerville (2014) also reported that smoking status was associated with adolescents who self-reported gender identity as homosexual/bisexual.

Cortisol AM and PM

In the current study, the morning cortisol levels (Cortisol AM) and afternoon cortisol levels (Cortisol PM) were within the normal range for adolescents of the age 14 to 16 years (Salimetrics, LLC, 2013). Females had significantly higher Cortisol AM as compared to males ($p < 0.003$).
Gunnar et al. (2009) also found cortisol levels were highest in 15 year olds as compared to 9, 11, and 13 year olds. In the current study, higher Cortisol AM and Cortisol PM, though not significant, was found to be associated with higher reports of stress (SLE, LCU) and bullying (PECK). Higher Cortisol AM, though non-significant, was also found to be associated with higher levels of perceived stress (PSS-10). Lower Cortisol AM and Cortisol PM, though not significant, was found to be associated with higher depressive symptoms.

Bosch et al. (2012) found when examining adolescents who had experienced adversity after the age of 11, there were lower levels of basal cortisol activity (hyposecretion) in comparison to higher levels of basal cortisol activity in children who had experienced adversity younger than age 11. These findings support the evidence that adolescents are sensitive to stressful experiences and HPA axis dysregulation during the major period of pubertal development. Lupien et al. (2009) discusses that cognitive (frontal cortex) and emotional processes in the brain are sensitive to cortisol in an age-related manner. Adolescents, who experience stress, will have higher HPA axis reactivity, which over time may then lead to stress-related mental disorders.

Netherton et al. (2004) found similar results to the current study with adolescents from 8 to 16 years old where cortisol AM was higher in mid-to post-pubertal females than in mid-to post-pubertal males. Gunnar et al. (2009) also found that females who had begun menstruation by the age of 13 had higher cortisol levels than males who were the same age. These results correspond to the current study in that pubertal status in those adolescents who were late-to post-pubertal also had higher cortisol levels than those adolescents who were early-to mid-pubertal.
Stressful Day

Adolescents were asked during the afternoon data collection period if something stressful had occurred that particular day; a stressful day was reported by less than one half (43%) of the sample. Stress during the day of the data collection was related to school issues as the major stressor, followed by peer issues, family issues, romantic issues, and personal issues. Adolescents who lack peer support often lack adult support (Langdon & Preble, 2008). During the 9th grade, adolescents experience increased academic responsibility and new adjustments to peer groups (Cushman, 2006). Barone et al. (1991) reported that 9th grade is associated with a lower grade point average (GPA) and lower attendance as compared to later years of high school. Although neither GPA nor attendance rate data was collected in the current study, it is important to recognize that one of the major stressors self-reported by 9th graders in the current study occurring on the day of data collection was related to academics or school issues.

Gender and race have been known to influence stress reactions during adolescence, though gender differences with stress reactivity have been inconclusive (Dowd, Palermo, Chuy, Adam, & McDade, 2014). Females tend to report higher levels of interpersonal stress and exhibit more emotional responses and stress reactivity (Jones, 1993; Rudolph, 2002). In the current study, more females reported a stressful day although the difference was non-significant as compared to males. White adolescents in the current study were more likely to report a Stressful Day as compared to Black/other racial minority adolescents, though these differences were not significant. There were also non-significant differences between the SES categories with regard to self-reporting a Stressful Day. Those who paid full price for lunch and those who brought lunch from
home reported a Stressful Day more often than those who received lunch for free or at a reduced cost.

In contrast, Dowd et al. (2014) found that Black adolescents had higher odds of stressful events occurring as compared to White adolescents and a strong association between having lower socioeconomic status and higher levels stressful events ($OR \ 7.47$, 95% CI 5.59-9.98). Although these results from Dowd (2014) study are in contrast to what was found in the current study, adolescents who did not have to pay for lunch nor had a lower cost for lunch may have perceived the situation at school as less stressful than those who had to pay the full price for lunch.

Adolescents whose gender identity was self-reported as homosexual/bisexual/or unsure reported a Stressful Day more frequently than those who were self-reporting as heterosexual. These findings are in line with the work of Reisner, Greytak, Parsons, & Ybarra (2015), which found that adolescents ($n = 5,907$) aged 13 to 18 who self-reported as homosexual or bisexual experienced fourfold higher odds of harassment than their heterosexual peers, which could be considered a stressful occurrence. In the current study, females self-reported a Stressful Day more than males, and the majority of females were late-to post-pubertal status, which also had more self-report of having a Stressful Day as compared to those who were mid-to late-pubertal status.

Findings Related to the Research Questions and Hypotheses

The purpose of this study was to examine stressful life events, perceived stress, and bullying to determine the influence of these variables independently and cumulatively on depressive symptoms. In addition, cortisol (physiological biomarker of
stress) was examined to determine if the cortisol diurnal rhythm has a mediating relationship between each of the independent variables (stressful life events, perceived stress, and bullying), and the dependent variable, depressive symptoms. The following section will discuss the findings of this study with regard to the research questions and hypotheses.

**Stress (Stressful Life Events, Life Change Units, and Perceived Stress)**

Adolescence as a developmental period is known to be associated with stress, and in this study, adolescents reported high levels of stress (SLE, LCU, PSS-10). In this study adolescents reported on average 14 stressful life events (range 0–79) and the majority of adolescents (80%) scored above the highest normative score for the LCU measure (score > 170). Perceived stress, the subjective evaluation of the stressfulness of a situation or perception of potential harm that could come from a stressor or stressful life event (Cohen et al., 1983), for the majority of adolescents in this study was in the middle range of the PSS-10 scores (10-30) with a small minority (2.8%) scoring in the top range, while only 13% had low PSS-10 scores (lower than 10). Adolescents who have high stress levels and a greater number of life changes are more likely to experience depressive symptoms (Martyn-Nemeth, Penckofer, Gulanick, Velsor-Friedrich, & Bryant, 2009).

In the current study females reported higher life change units (LCU $\bar{x} = 420$) and significantly higher scores for perceived stress (PSS-10 $\bar{x} = 20$) as compared to males (LCU $\bar{x} = 387$, PSS-10 $\bar{x} = 17$). These findings are in agreement with Newcomb et al. (1981) who found that there were certain stressful events where females scored significantly higher than males. For example, females self-reported more parental divorce, finding a new group of friends, falling in love, becoming pregnant, or thinking
about suicide; whereas, males scored significantly higher on events that resulted in deviant behavior. Similar findings are seen in many other studies (Aro, Hanninen & Paronen, 1989; Jones, 1993; McKay, Dempster, & Byrne, 2014; Villalong-Olives et al., 2011).

In contrast the study findings, Austin, Smith, and Patterson (2009), found that adolescent males had significantly higher PSS-10 scores than females. Thaker and Verma (2014) found similar results with males scoring significantly higher than females on the PSS-14 scale (males- $\bar{x} = 42.58$, $SD = 5.94$, females - $\bar{x} = 38.48$, $SD = 5.29$). In the current study White adolescents had higher in SLE and LCU, which is contrary to what is seen in the literature; however, there were no differences for PSS-10 scores. In contrast to the current study, Wickrama, Lee, and O’Neal (2015) found that adolescents who were Black or Hispanic were more likely to experience higher adversity or SLE than their Caucasian peers. Goodman et al. (2005) found that Black adolescents had higher PSS-14 scores than White adolescents ($\beta_{black} = 1.82$, SE = 0.62, $p = 0.004$).

Finkelstein, Kubzansky, Capitman and Goodman (2007) found that non-Hispanic Blacks had higher PSS-14 scores than non-Hispanic Whites ($p < 0.0001$). There are many other studies that report race, particularly minority status, as strongly correlated to stressful life events and the consequences that are related to these events (Baldwin, Harris, Chambliss, 1997; Dowd et al., 2014; Goodman, McEwen, Dolan, Schafer-Kalkhoff, & Adler, 2005). There may be other factors influencing the stress levels in White adolescents such as economic, academic, extra-curricular activities, and environmental issues.
In the current study, adolescents with lower income (free or reduced-cost lunch) had higher levels of SLE, LCU, and PSS-10, though these differences were not significant. These findings are similar to what is found in the literature. Gundy et al. (2015) found adolescents with higher SES, reported fewer stressful life events as compared to adolescents with lower SES. In a review of research, Hatch and Dohrenwend (2007) examined stressful life events and socioeconomic status and found conclusively that lower SES individuals had more stressful life events. These researchers explain that there may be limited opportunities and fewer resources for the lower SES individuals as compared to higher SES individuals.

Many other studies were found that examined socioeconomic status and stress in adolescents that indicated the same results (Finkelstein et al., 2007; Sweet, Nandi, Adam, & McDade, 2013; Sweet, 2010; Van Gundy et al., 2015). Constant exposure to stressful circumstances usually include issues such as lack of health resources, poor educational attainment, lack of recreational activities, and all of these issues related to stressful life events have an impact (Bae, Wickrama, & O’Neal, 2014).

In the current study, adolescents who self-reported as homosexual, bisexual or unsure of their gender identity reported higher SLE ($\bar{x} = 18$), significantly higher LCU ($\bar{x} = 577$), and significantly higher PSS-10 ($\bar{x} = 24$) as compared to their self-reporting heterosexual peers (SLE $\bar{x} = 13$, LCU $\bar{x} = 379$, PSS-10 $\bar{x} = 18$). Similar to these findings, Hatzenbuehler, Slopen, and McLaughlin (2014) examined sexual minority adolescents ($n = 306$) and found that adolescents who are in the sexual minority (homosexual, bisexual, or transgender) are more vulnerable and had higher stress scores as compared to their heterosexual peers. Berlan, Corliss, Field, Goodman & Austin (2010) asked adolescents
aged 14 to 22 years to report their gender identity out of a group of five different categories. For males \((n = 2,720)\), 93.5% described themselves as heterosexual; however, 4.5% described themselves as mostly heterosexual, 0.5% as bisexual, and 1.4% as mostly homosexual or completely gay. For females \((n = 4,839)\), 88.3% described themselves as heterosexual, 9.5% as mostly heterosexual, 1.9% as bisexual, and only 0.3% as mostly homosexual or completely lesbian.

Ueno (2005) examined sexual orientation and psychological distress in adolescents \((n = 200)\) aged 13 to 19 and found that adolescents in the sexual minorities were more likely to have trouble at school with peers and teachers. Furthermore, sexual minority adolescents were more likely to have arguments with parents than heterosexual adolescents. In a meta-analysis by Collier et al. (2013), findings included evidence that sexual orientation and gender identity issues are associated with stress from traumatic events. In addition, Balsam et al. (2011) discusses that adolescents who struggle with their gender identity may suffer three types of stressful micro aggressions including: micro assaults (discrimination), micro insults (discrediting an individual due to societal beliefs), and micro invalidation (individual is excluded or experiences are negated).

In the current study, adolescents who self-reported late-to post-pubertal status had significantly higher SLE scores \((\bar{x} = 16)\), LCU scores \((\bar{x} = 445)\), and PSS-10 \((\bar{x} = 20)\) scores as compared to those adolescents who were early-to mid-pubertal status \((SLE \bar{x} = 11, LCU \bar{x} = 326, \text{PSS-10} \bar{x} = 17)\). In a review article, Holder & Blaustein et al. (2014) discuss that the experience of puberty generally increases reactivity to stress; therefore the hormones, such as estrogens and progestin in females and androgens in males can contribute to this sensitivity to stress.
Ge, Conger, and Elder (2001) examined stressful life event and found that there were significant gender differences from mid-to late-adolescence. These authors suggest that adaptation to early puberty, especially when other environmental stressors are occurring is particularly difficult for females. Dahl and Gunnar (2009) discuss that as pubertal status progresses there are several things that typically take place that can be related to stress: sexual maturation, romantic and sexual interests, risk taking or reckless behaviors, self-consciousness, alcohol and substance abuse, increase in accidents and suicidal ideation.

A study by van Jaarsveld, Fidler, Simon, and Wardle (2007) found that in adolescents (n = 5,863) age 11 to 16, there was no significant difference in PSS-4 scores among males in the different pubertal groups (early, average, or late maturing); however, there was higher perceived stress in both early and late maturing females as compared to the average maturing females (OR = 1.49, 95% CI = 1.19; 1.88).

Cortisol Diurnal Rhythm

Cortisol diurnal rhythm was measured by the cortisol change in the morning to the afternoon levels with a mean of -0.07 (SD = 0.146). In this study, the majority of adolescents (64.3%) had cortisol diurnal rhythms that followed the expected pattern of being higher in the morning and slowly decreasing throughout the day. There was a small group (12.5%) who had a flattened or blunted cortisol diurnal rhythm (less than 0.01 µg/dL from AM to PM), and another group (23%) that had an opposite diurnal rhythm with lower cortisol in the morning and higher cortisol in the afternoon.

The results of the current study with regard to cortisol diurnal rhythm were similar to those of Shirtcliff et al. (2012), who found that as children progressed into
adolescence and became more physically developed, they had higher cortisol levels and a flatter circadian rhythm. Gunnar et al. (2009) posits that these results may be due to the amount of stress experienced due to the emotional and cognitive transition of this developmental period. Rahdar & Galvan (2014) studied adolescents aged 15 to 17 ($n = 22$) and adults aged 25 to 30 ($n = 23$). Results indicate that in both adolescents and adults, daily stress impairs inhibition response; however, the effect was stronger in adolescents as compared to adults.

Females are known to have higher cortisol levels and larger cortisol diurnal rhythm change as compared to males (Rosmalen et al., 2005). This was confirmed in the current study, as females ($\bar{x} = -0.09$) had a significantly larger cortisol change from AM to PM (cortisol diurnal rhythm) than males ($\bar{x} = -0.04$); however, the assumption of homogeneity was not met, so these results should be viewed with caution. Cortisol levels are known to vary in adolescent females who are taking birth control pills, who are pregnant, or who are breast feeding, as well during the menstrual cycle phase (Kudielka, Hellhammer & Wust, 2009). Shirtcliff et al. (2012) found females having higher cortisol levels overall, with steeper slopes and more curvature of the rhythm than males.

Puberty is thought to be a period of time when there may be changes in the cortisol regulation (HPA axis) (Dorn, Dahl, Woodward, & Brio, 2006), especially in adolescent females (Dahl, 1992; Keenan et al., 2013; Netherton, 2004; Tornage, 2002). In the current study, adolescents who self-reported late-to post-pubertal status (-0.09) had a significantly larger range of change from AM to PM cortisol as compared to adolescents who self-reported as early to mid-pubertal status (-0.03). Changes in the HPA
axis and the differences in cortisol levels in gender occurring during puberty may continue through adolescence and into adulthood (Netherton et al., 2004).

In contrast to the current study, Knutsson et al. (1997) found that the cortisol diurnal rhythm was “extremely robust” (p. 539) and did not appear to be related to age, gender, or pubertal status, though there were significant inter-individual variations. Rotenberg, McGrath, Roy-Gagnon, and Tu et al. (2012) posits that cortisol basal rates as well as cortisol diurnal rhythm is consistently related to adrenarche (the period of development from age 5 to age 20) as compared to gonadarche (the initiation of production of sex steroids).

Berger and Sarnyai (2015) discuss that chronic stress can be caused by discrimination, which can lead to an altered stress response (cortisol diurnal rhythm). In the current study there was no difference in cortisol diurnal rhythm for Black/other adolescents ($\bar{x} = -0.08$) as compared to the White adolescents ($\bar{x} = -0.07$). In contrast, other studies reported that Black adolescents had flatter diurnal cortisol rhythms as compared to White adolescents (DeSantis et al., 2007; Martin, Bruce and Fisher (2012). Other factors than race may explain changes in cortisol diurnal rhythm.

In the current study those adolescents who paid full price for lunch or brought lunch from home ($\bar{x} = -0.08$) had a slightly larger change in the cortisol diurnal rhythm; although this difference was non-significant when compared to those adolescents who received the free/reduced-cost lunch ($\bar{x} = -0.06$). Similarly, DeSantis et al., (2007) found no difference in SES, though lower SES had a flatter cortisol diurnal rhythm change. In another study, Sieh, Visser-Meily, Oort, and
Meijer (2012) found no significant differences in cortisol diurnal rhythm in relation to socioeconomic status (measured by average monthly income).

In the current study, there was a larger change in the cortisol diurnal rhythm in the homosexual/bisexual/unsure adolescents ($\bar{x} = -0.011$) as compared to those adolescents who reported being heterosexual ($\bar{x} = -0.07$), but these differences were non-significant ($p = 0.403$). Juster et al., (2014) reported no differences in cortisol diurnal rhythm by gender identity. There were small cell size for homosexuals, bisexuals or those who were unsure of their gender identity.

**Bullying (PECK)**

Adolescent reports of bullying (PECK scores) in this study were lower than expected ($\bar{x} = 15$, $SD = 15$, range 0-98) as was also found in the pilot study ($\bar{x} = 12.9$, $SD = 11.36$, range 0 – 48). Bullying rates in the current study were similar to that of the Fredstrom et al. (2010) study that had similar population demographics to the current study population. In the original PECK study, Hunt et al., (2012) reported approximately 21% of the sample ($n = 647$) adolescents (8-15 years) had bullying (2 or 3 times per month). This level of two to three times per month was recommended as the lower bound cut off from the Olweus Bullying Victim Questionnaire (OBVQ) (Solberg & Olweus, 2003). Using these criteria in the current study, approximately 21% of the sample met criteria for significant bullying (PECK score 16-30), and 13% of the study sample scored higher (scores from 31 to 100) on the PECK survey. The current study found similar results as Bogart et al. (2014) who reported 30% of adolescents ($n = 4,297$, 5th to 10th grade) reporting frequent bullying experiences.
The types of bullying in this study were defined as verbal/relational, physical, cyber, and cultural. The PECK score ranges from 0-128, with subscale scores representing the types of bullying. Individual types of bullying for this study were as follows: verbal/relational (standard range: 0-44; current study: 0-30), physical (standard range: 0-36; current study: 0-21), cyber (standard range: 0-32; current study: 0-28), and cultural (standard range: 0-16; current study: 0-15). While there were a few 9th graders who had scores reaching close to the top of the range in each specific bullying type, the mean in each category was low. Individual bullying type mean scores were: verbal/relational, ($\bar{x} = 8$), physical ($\bar{x} = 2$), cyber ($\bar{x} = 8$), and cultural ($\bar{x} = 8$).

Approximately 11% of the 9th graders in the current study were bullied by verbal/relational means. As was also seen in the Hunt et al. (2012) study, most bullying occurred in the verbal/relational bullying category. This type of bullying was self-reported as happening sometimes, most days, or every day. When evaluating the other types of bullying, the self-report scores were all below the “sometimes happening” level. In the current study, Black and other racial minority adolescents had significantly higher cultural bullying ($\bar{x} = 2.0$) and also higher but non-significant differences in cyberbullying ($\bar{x} = 2.2$) as compared to White adolescents (cultural bullying, $\bar{x} = -0.07$, cyberbullying $\bar{x} = 1.7$).

Goldweber et al. (2013) examined bullying in adolescents ($n = 10,254$) and found that one-third of adolescents reported being bullied about the way they talked, looked, or dressed. The majority of the sample was White (62.5%); however, the other adolescents were either Black (19.1%), or other races (18.4). The next most common reason cited in the Goldweber et al. (2013) study for being bullied was related to race or skin color.
Blacks were significantly more likely to be in the “high involvement” category of bullying \((p < 0.05)\) (Goldweber et al., 2013, p. 479). Low and Espelage (2013) examined adolescents \((n = 1,023)\) and found that Black adolescents self-reported higher levels of cyberbullying as compared to White adolescents.

The current study found no significant differences in bullying as related to socioeconomic status. In contrast, Tippett & Wolke (2014) found that the lower SES population were more likely to be victims of bullying as compared to those who were from higher SES households \((OR = 1.40, 95\% CI = 1.24, 1.58)\). In another study involving 35 countries and adolescents aged 11 to 15 years \((n = 162,305)\), Due et al. (2009) found that adolescents from lower SES households had higher odds of reporting bullying victimization as compared to those adolescents who were from higher socioeconomic status households \((OR = 1.13, 95\% CI 1.10, 1.16)\).

In the current study, adolescents who self-reported as homosexual/bisexual or unsure of their gender identity had significantly higher verbal relational bullying scores \((\bar{x} = 14)\) and higher though not significant rates of cyberbullying \((\bar{x} = 1.6)\) as compared to adolescents who self-reported as heterosexual \((\text{verbal/relational bullying } \bar{x} = 7.0, \text{ cyberbullying } \bar{x} = 1.0)\). Robinson et al. (2013) studied gender identity and bullying in adolescents \((n = 187)\) and found that 57% of lesbian/bisexual females and 52% of males self-reported being bullied in the 9th grade. Berlan et al. (2010) studied sexual orientation and bullying among adolescents \((n = 7,559)\) aged 14 to 22. Homosexual males (relative risk \([RR] 1.98; 95\% CI: 2.39, 2.82)\) were more likely to report being bullied as compared to their heterosexual peers \((RR: 1.45; 95\% CI: 1.13, 1.86)\). Bisexual females \((RR: 1.63,\)
95% CI: 1.14, 2.31), lesbians (RR: 2.41, 95% CI: 1.80, 3.24) were more likely to report being bullied than their heterosexual peers (RR: 1.70, 95% CI: 1.42, 2.04).

Marshal et al. (2011) found that those adolescents who are in the sexual minority have more mental health problems as compared to their heterosexual peers. In addition, the sexual minority population has higher rates of suicidal ideation and suicide attempts. Adolescents in the current study who self-reported late to post-pubertal status had higher though not significantly different PECK scores in comparison to those who self-reported early to mid-pubertal status. In a Chinese study by Tam and Zhang (2012), research suggests that developmental changes, particularly in females experiencing puberty may be a risk factor for bullying. When searching the literature for articles specifically pertaining to bullying and pubertal status, there were very few available. The majority of the articles available were related to middle school and not high school adolescents.

**Depressive symptoms (CESD-10)**

The current study’s responses from the CESD-10 had a high percentage (38%) of clinically significant depressive symptoms (CESD-10 score > 9). Martyn-Nemeth and colleagues (2009) found that increased stress had a significant direct effect on depressive mood (β -.52, p < 0.001) in a diverse sample of adolescents aged 14 to 18 (n = 102). Similar results were found in the pilot study where the PSS-4 was used and adolescents who had a high PSS-4 score also had a high CESD-10 score.

In the current study, females had higher CESD-10 scores than males; however, these differences were non-significant. Sangalang and Gee (2015) had similar finding in that females scored higher yet non-significant differences in depressive symptoms as compared to males. Penden et al. (2005) studied adolescents aged 14 to 18 (n = 299),
finding the prevalence for significant depressive symptoms (CESD-20 scores >16) in this population was 34%. The reports of depressive symptoms in males were similar to those reported by females, in contrast with other studies of depressive symptoms that showed that females reported more symptoms than males (Paredes & Zumalde, 2014; Tummala-Narra, 2015; Weeks et al., 2014).

Sheriff, McGorry, Cotton, & Yung (2015) conducted a qualitative study with adolescents \( (n = 29) \) ages 15 to 18 years to uncover the gender differences in the prodromal period or early depressive symptoms. All adolescents experienced a prodromal period between 6 days to four years with common symptoms of confusion, sadness, and irritability followed by inability to conduct their daily routine, along with feelings of guilt, lower self-esteem and blaming of self. Males noticed a change in how they related to the world around them, whereas females noticed a change in the way they related to the individuals around them.

Lewis et al. (2015) examined differences in depressive symptoms in adolescents aged 10 to 14 years \( (n = 6,552) \). Specific differences included females who had low emotional closeness to their parents were 2.3 times more likely to have high depressive symptoms than females who had high emotional closeness \( (OR = 2.34, CI = 1.71-3.19, p < 0.01) \) Approximately 22% of males with low emotional closeness met criteria for severe depressive symptoms as compared to 18% meeting criteria for severe depressive symptoms despite high emotional closeness to parents. Hall et al. (2013) examined depressive symptoms in a group of late-adolescent females aged 18-20, and results indicated that 27% of females met criteria for severe depressive symptoms (score > 4 on the CESD-5).
In the current study, White adolescents had higher yet non-significant differences in CESD-10 scores than Black/other adolescents. Raffaelli and colleagues (2013) examined gender and age-related differences in Mexican adolescents \((n = 5,152, \text{ages } 17-20)\) and found significantly more females (28%) reported depressive symptoms than males (18%) \((p < 0.001)\). Tummala-Narra (2015) examined 9th and 10th grade adolescents \((n = 341)\) and found that ethnicity and race were negatively associated with depressive symptoms. All adolescents were of Asian, Latino, or Afro-Caribbean racial/ethnic minority background. Those adolescents who were born in the U. S. had higher depressive symptoms than those who were born in their Native country \((b = -0.107, p < 0.05)\).

Sangalang and Gee (2015) examined Cambodian-American adolescents \((n = 466)\), aged 13 to 19, and found that depressive symptoms were correlated with racial discrimination \((r = 0.42, p < 0.001)\), peer discrimination \((r = 0.29, p < 0.001)\), school discrimination \((r = 0.35, p < 0.001)\), and police discrimination \((p = 0.34, p < 0.001)\). In the current study, CESD-10 scores were slightly higher but not significant for adolescents who receive free/ reduced-cost lunch than those who pay full price lunch status or bring lunch from home. When evaluating other studies with regard to depressive symptoms and socioeconomic status, other issues besides financial status are usually considered when examining depressive symptoms. These issues include but not limited to parental education, neighborhood characteristics, racial/ethnic composition of community, and availability of treatment for mental health issues like depression (Cummings, 2014).
Jeon et al. (2013) found lower socioeconomic household status reported more depressive symptoms, and suicidal ideation. Similarly, Mistry et al. (2009) reported findings in a Chinese-American study with family economic strain predicting depressive symptoms in later adolescence. Kubik et al. (2003) found that socioeconomic and minority status was independently related to depressive symptoms. Over 40% of low SES adolescents reported depressive symptoms when compared to 25% of adolescents who were in the category of high SES. Goodman et al. (2003) also found socioeconomic status and lower parental education influenced mental health with one third of depression reported in adolescent with lower household incomes and lower parental education. Nebbitt and Lombe (2007) found a significant relationship between socioeconomic status and depressive symptoms particularly with Black adolescent males living in public housing developments.

In the current study there were significant gender identity differences in the depressive symptom scores with homosexual/bisexual/unsure adolescents scoring higher on the CESD-10 ($\bar{x} = 15$) as compared to the heterosexual adolescents ($\bar{x} = 7.9$). While the literature is clear in that sexual orientation is the term describing preference of heterosexual, homosexual, bisexual, or unsure (American Psychological Association, 2011), the term “gender identity” was used in the current study to survey the adolescents with regard to self-report as heterosexual, homosexual, bisexual, or unsure. This was a strong recommendation of the school principal.

Marshal et al. (2013) examined sexual minority adolescents ($n = 12,379$) with results indicating that depressive symptoms were significantly higher among those adolescents self-reporting as gay, lesbian, mostly heterosexual, and bisexual as compared
to those who were heterosexual ($\mu_a = -0.085, SE = 0.017, p < 0.001$). In contrast to the current study, Weber (2009) found that reporting of depressive symptoms was similar between self-identified heterosexual and those with gender identity developmental issues (self-identified as gay, bisexual, or transgender) when comparing adolescents in a urban school sample ($n = 357$) aged 13 to 18. In another study, perceived interpersonal discrimination was associated with depressive symptoms in sexual minority youth ($\beta = 0.39, SE = 0.08, p < 0.001$) as well as heterosexual youth ($\beta = 0.28, SE = 0.04, p < 0.001$).

In the current study, adolescents who were late-to post-pubertal status had higher depressive symptom scores, though not significantly, than those who were early-to mid-pubertal status. Conley and Rudolph (2009) examined depressive symptoms in adolescents. When looking at males as compared to females, males who had later pubertal timing had higher levels of depression ($\beta = 0.34, p < 0.05$), than females who higher levels of depression and earlier pubertal timing ($\beta = 0.34, p < 0.05$).

Ge et al. (2001) conducted a longitudinal study in adolescent males 7th grade until 10th grade ($n = 406$) and results showed an association between pubertal status and internalized distress and hostile feelings over a four year period. In 8th grade and in 9th grade the correlation was between pubertal status and hostility (8th grade, $r (1) = 0.24, p < 0.01$, 9th grade $r (1) = 0.18, p < 0.05$). When examining 7th grade males, the correlation was significantly related to internalizing distress ($r (1) = 0.23, p < 0.01$ and hostility ($r (1) = 0.26, p < 0.01$). These findings suggest that pubertal status is related to emotional and behavioral adjustment.
Correlations among Variables

The second research question/hypothesis examined correlations among the independent and dependent variables. The hypothesis was partially supported in that there were significant positive correlations with SLE ($r_s = 0.35$), LCU ($r_s = 0.42$), PSS-10 ($r_s = 0.73$), and PECK ($r_s = 0.65$) ($p < 0.01$ for SLE, LCU, PSS-10 and PECK) and depressive symptoms (CESD-10). There was not a significant correlation, however, between cortisol diurnal rhythm and CESD-10. This discussion includes the magnitude of the relationship and the effect size using Cohen’s standard (small magnitude = 0.10–0.20, medium magnitude = 0.30–0.49, large magnitude = $> 0.50$) (Leech, Barrett, & Morgan, 2011).

Kaplan et al. (1986) found a significant relationship between undesirable life events and depressive symptom scores using the Coddington Life Event Survey and the Beck Depression Inventory ($r = 0.44$, $p < 0.01$). Murray, Rieger, & Byrne (2013) found significant correlations between stress and depressive symptoms for males ($r = 0.49$, $p < 0.01$) and for females ($r = 0.66$, $p < 0.01$). Hall et al. (2013) study found that depressive symptoms and stress were significantly correlated, and Zhang, Yan, Zhao & Yuan (2014) found similar results when examining perceived stress and depressive symptoms in adolescents with a significant correlation ($r = 0.31$, $p < 0.01$). Hankin, Fraley, and Abela (2005) found a significant correlation between the Beck Depression Inventory and a stress reaction subscale ($r = 0.57$, $p < 0.01$) when examining undergraduate college students ($n = 217$).

Lupien, McEwen, Gunnar, and Hein (2009) discuss that many times, a period of stress will occur before the onset of depressive symptoms or other psychiatric disorders.
in adolescence. Furthermore, adolescents have a heightened HPA axis reactivity that makes them susceptible to having mental health disorders. These researchers discuss that another area affected by stress during adolescence is the pre-frontal cortex that is undergoing major development. When an adolescent is under stress, there is vulnerability due to a protracted glucocorticoid response that may persist into adulthood causing potentiation and incubation effects.

In the current study, both school-based bullying and bullying through technology (cyber) were significantly correlated with depressive symptoms ($r = 0.23, p < 0.01$). The current study also showed significant correlations with depressive symptoms and each type of bullying ranging from verbal/relational ($r_s = 0.61, p < 0.01$, large magnitude), cyber ($r_s = 0.35, p < 0.001$, small magnitude), physical ($r_s = 0.49, p < 0.001$, medium magnitude), and cultural ($r_s = 0.31, p < 0.001$, small magnitude).

Similar to the current study where depressive symptoms were correlated with bullying, 12% of adolescents in the Bogart et al. (2014) study who were bullied in the past had low psychological health. Cole et al. (2014) also found similar results as the current study as relational/verbal victimization was positively correlated with negative self-cognitions. Although the children were younger in the Cole et al. (2014) study, evidence points consistently to verbal and relational bullying being more “toxic” than physical bullying when examining depressive symptoms in a population of children or early adolescents.

Even though cyberbullying scores were low ($\bar{x} = 2, SD = 3$, range = 0-28) in the current study, this form of victimization was correlated with depressive symptoms. Therefore, monitoring this type of bullying is important. Elgar et al. (2014) examined
cyberbullying as it relates to mental health and substance use among adolescents and found that 18% of a sample of adolescents \((n = 18,834)\) aged 12 to 18 had experienced cyberbullying over the previous year. Cyberbullying was positively related to 11 different internalizing, externalizing, and substance use problems \((OR = 2.6 \ CI, \ 1.7-3.8-4.5, \ 95\% \ CI, \ 3.0-6.6)\).

Gamez-Guadiz, Orue, Smith, & Calvette (2013) examined cyberbullying and depressive symptoms in adolescents \((n = 845)\), mean age of 15.2 at two time points, six months apart. Cyberbullying was correlated with depressive symptoms at Time 1 and six months later at Time 2. In addition, results indicated that cyberbullying predicted depressive symptoms; these researchers also found that there was a reciprocal relationship in that depressive symptoms could also lead to cyberbullying. Gamez-Guadiz et al. (2013) posit that this reciprocal relationship may occur due to adolescents’ having poor social skills that may cause them to become isolated. In turn, cyberbullying can cause an adolescent to feel lonely, to have poor social and personal relationships, and result in feelings of sadness and rejection.

Females tend to show increased sensitivity to environmental or family problems. This along with the increased gonadal steroids during menarche and increased glucocorticoids during stress is known to be related to depressive disorders (Lupien et al., 2009). Gamez-Guadiz et al. (2013) found that female gender was correlated with depressive symptoms at both time points in their study with adolescents.

Race was found to be significantly correlated with cultural bullying \((r = -0.242, \ p = 0.004, \ small \ magnitude)\) in the current study. A similar result was found with Espinoza, Gonzales, & Fuligni (2014) in a study examining Mexican-American 9th and 10th graders
Twenty-five percent of Mexican-American adolescents reported a minimum of one verbal or physical bullying experience over a two week period with no gender differences. Third generation Mexican-Americans received more bullying than 1st generation Mexican-Americans. Espinoza et al. (2013) posited that third generation Mexican-Americans may place more emphasis on peer friendships, therefore, have more peer exposure and targeting of bullies. First generation immigrants may be in tighter knit family-oriented groups. These adolescents in first generation may be more protected from victimization than 3rd generation Mexican-Americans.

When examining correlations with the covariates, the one covariate that stood out as being correlated with each of the major independent variables and the dependent variable was gender identity. This finding was also seen in the literature. Williams, Conolly, Pepler & Craig (2005) examined sexual minority (gay, lesbian, bisexual, and those who were unsure of their sexual identity) adolescents ($n = 97$) and their heterosexual peers ($n = 97$). Sexual minority adolescents reported more depression ($p < 0.01$), and more bullying ($p < 0.05$) than their heterosexual peers.

These correlations of gender identity, with depressive symptoms, as well as stressful life events, life change units, perceived stress, and bullying, most specifically cyberbullying, bring to the forefront that there are many risks and difficulties that an adolescent who is in the sexual minority faces on a daily basis. Being aware of this population, reaching out to adolescents who are struggling, and developing policies and procedures to help protect this vulnerable population may change the trajectory of an individual’s life both in high school and long after high school is completed (Konishi et al., 2013).
Jin et al. (2008) had similar findings in a longitudinal study with Black children \( (n = 890) \) who were studied from the age of 10 to 12 years until these children reached the ages of 16 to 18 years. Children with internalizing disorders had earlier onset of puberty \( (p < 0.05) \), a higher tendency for risk taking \( (p < 0.01) \), and more stressful life events \( (p < 0.001) \) as compared to those children who had no internalizing disorders. The finding of the current study seem to differ from these findings in that the later pubertal status scores were associated with more stressful life events, higher scores on life change units, and higher perceived stress.

When conducting a longitudinal study, Benoit, Lacourse and Claes (2013) found that earlier pubertal timing was significantly associated with higher levels of depressive symptoms \( (\beta = 0.08, p < 0.05) \). In addition, having an earlier dating experience \( (\beta = 0.10, p < 0.01) \) and having a perception of being less popular \( (\beta = -0.12, p < 0.001) \) was significantly associated with depressive symptoms. Even though there was no significant pubertal status difference in the current study with regard to depressive symptoms, it is important to be aware that these hormonal changes, especially in females, when occurring at an early age, and when combined with emotional problems in the later stages of childhood, can put an individual at risk for depressive symptoms.

Hamlat et al. (2014) conducted a study with White and Black adolescents \( (n = 218) \) with a baseline assessment and a second assessment 8 months later to examine pubertal timing, peer victimization, body esteem, and depressive symptoms. Results showed that White females who matured early and Black females who matured later had more depressive symptoms if they also experienced peer victimization between the two
time points. Pubertal timing nor peer victimization were not predictors of depressive symptoms in White or Black males.

There were no significant correlations between cortisol diurnal rhythm and depressive symptoms; however, pubertal status was negatively correlated with cortisol diurnal rhythm in the current study \((r_s = -0.281, p = 0.001, \text{small magnitude})\). A similar result was found in a study by Saczawa, Graber, Brooks-Gunn, and Warren (2013) who examined the individual cortisol levels and pubertal status. Results included negative correlations between cortisol slope and intercept \((r = -0.66, p < 0.05)\), and between Tanner PH (pubertal status score) intercept and slope \((r = -0.17, p < 0.001)\). In the Saczawa et al. (2013) study, the cortisol levels were measured over a three year period, and the adolescents who began the study with the highest cortisol levels were also the most developed with regard to pubertal status. This group of adolescents also had the least change in their cortisol levels over time.

Rotenberg et al. (2012) conducted a study examining diurnal cortisol levels in children and adolescents \((n = 233)\) aged 9 to 18 years by collecting cortisol levels five times per day for 3 days at home or at school. Pubertal status based on gonadarche (physical and sexual maturation) was not significantly associated with any of the cortisol levels that were measured. Pubertal status based on adrenarche or adolescents that had pubic hair development had significantly flatter diurnal slopes.

**Variance in Depressive Symptoms**

The third research question and hypothesis were focused on how much variance in depressive symptoms was accounted for by each independent variable (stressful life events, life change units, perceived stress, and bullying) and the mediating variable
(cortisol diurnal rhythm). Perceived stress (large effect size) and bullying (small effect size), and one covariate, gender identity (small effect size) contributed 67% (p < 0.05) of the variance in depressive symptoms. As an independent variable, perceived stress contributed the largest variance (48%) in depressive symptoms. Cortisol diurnal rhythm had no relationship with depressive symptoms (p > 0.05). Thus, the variables that contributed the most to depressive symptoms were perceived stress, bullying and gender identity; the third hypothesis was partially supported. Cumulatively there was a significant percentage of the variance in depressive symptoms explained by the combination of two independent variables (PSS-10 and PECK) after controlling for gender, race, gender identity, socioeconomic status (SES), and pubertal status.

The first model, with the five covariates, was significant for predicting depressive symptoms; however, the only significant variable out of this model was gender identity that contributed negatively toward depressive symptoms. That is, those adolescents who self-reported being homosexual, bi-sexual, and those unsure of their gender identity had higher LCU, PSS-10, PECK, and verbal/relational bullying and CESD-10 scores than heterosexuals.

In the second model, the independent variables of perceived stress and bullying were the only significant contributors to the total variance in depressive symptoms. Gender identity remained a significant contributor to the depressive symptoms scores in the fourth model. Burton et al. (2013) studied adolescents aged 14 to 19 (n = 197) and found that sexual minority youth (homosexual/bisexual or other) reported higher levels of sexual minority victimization, depressive symptoms, and suicidality with correlations to sexual minority victimization and depressive symptoms (r = 0.66, p < 0.001).
Although the current study found that stressful life events (SLE) and life change units (LCU) did not contribute significantly towards the variance in CESD-10 scores, Hankin et al. (2005) found that negative life events accounted for 41.76% of the variance in daily depressive symptoms. Meadows et al. (2006) found that stressful life events predicted depressive symptoms in females in three waves of data from 1994 – 1996 (Wave 1, β = 0.24, p < 0.001; Wave 2-β = 0.42, p < 0.001; Wave 3, β= -0.31, p < 0.01); whereas, for males’ depressive symptoms were only predicted at the second wave of data (β = 0.17, p < 0.001). In the current study, perceived stress appears to be a higher predictor of depressive symptoms (β = 0.454, p < 0.05) than what is seen in the literature discussed above.

When examining how bullying was related to depressive symptoms, verbal relational bullying scores were highest overall, with physical bullying as second highest. Males had higher significantly higher physical bullying scores than females in the current study and also were higher in verbal-relational bullying. Females had higher though not statistically different CESD-10 scores (x̄ = 9.1) as compared to males (x̄ = 8.7). Cyberbullying scores were very low overall for adolescents in this study. Higher bullying scores resulted in higher depressive symptom scores for males and for females.

In a Finnish study on bullying during adolescence, Kaltiala-Heino et al., (2000) reported low rates of weekly bullying (females, 5%, males, 6%). Of those who experienced bullying, 25.5% of females self-reported depressive symptoms as compared to 15.7% of males when controlling for age, sex, family structure and parent education. Females (11%) had significantly higher depressive symptoms as compared to males (6%).
Kaltiala-Heino et al. (2000) stated that bullying/victimization was important to recognize as those adolescents are more likely to be at an increased risk for mental health disorders. Other factors, such as a school climate, may influence bullying in schools. Mehta et al. (2013), in a study of 9th graders \( n = 7,058 \), 289 schools found that bullying climate was correlated with victimization \( r = 0.347, p < 0.01 \) at an individual level. While the current study did not measure school climate, this variable may be important.

In a study with 9th – 12th graders \( n = 20,406 \), Schneider et al. (2012) found that 15.8% of adolescents reported cyberbullying, and 25.9% reported school bullying experiences in the last 12 months, with the majority of cyberbullying victims also reporting school bullying. Those adolescents who were non-heterosexual self-reported higher victimization rates than heterosexuals. Cornell et al. (2013) studied 9th graders \( n = 276 \) in public high schools to determine if teasing and bullying were predictive of dropout rates in high school. Results indicated that those adolescents who bullied others and those who are also victimized (bully/victims) had significant correlations with student dropout rates in several categories: student-reported general victimization \( r = 0.15, p < 0.001 \), teacher-reported teasing and bullying \( r = 0.33, p < 0.001 \), and student reported teasing and bullying \( r = 0.32, p < 0.001 \). When binomial regressions were run, student-reported perceived teasing and bullying accounted for 21% of the variance toward the dropout counts.

Langdon and Preble (2008) examined 5th–12th graders \( n = 3,147 \) with results showing that approximately 15% of adolescents self-reported observing physical bullying at least weekly, and 12% self-reported being picked on daily. The following groups
reported higher amounts of bullying and lower levels of respect: males, minorities, 9th graders, 10th graders, and non-college bound adolescents.

The above studies on bullying found a range of bullying (5%–26%) in 9th grade students, which is a broader range of bullying than found in the current study (21%–33%). The 9th grade has been described as a “linchpin year” (Donegan, 2008), meaning that having a successful first year in high school is important to success in school in the future. Several of the above studies also found correlations with depressive symptoms, especially in populations identified as sexual minority, females, racial minority, and those not involved in extracurricular activities. These particular groups of 9th graders deserve careful attention to help ensure their mental health remains stable during this first transitional year (Donegan, 2008).

Cortisol as a Mediator

While there are multiple studies that equate elevated cortisol levels with depressive symptoms (Bhagwagar et al., 2005; Burke et al., 2005; Fernald et al., 2008; McCabe & Schneider, 2009; Miller et al., 2007), the mediator hypotheses examining the relationship of cortisol diurnal rhythm on the independent variables (stressful life events, life change units, perceived stress, bullying) and dependent variable (depressive symptoms) were not supported. In this study, cortisol diurnal rhythm was not correlated with depressive symptoms on any level, even when putting depressive symptoms into categories of low, medium, and high scores. However, diurnal cortisol was correlated with pubertal status ($r_s = 0.193, p = 0.022, \text{small magnitude}$) and gender ($r_s = -0.167, p = 0.047, \text{small magnitude}$).
Smyth et al. (1997) described results of a diurnal cortisol study that was completed over a two day period. This author identified three possible subgroups when analyzing the diurnal rhythm of cortisol: 1) typical cortisol peak and decline throughout the day; 2) a flat diurnal group where evening cortisol levels fail to decline and remain elevated; and 3) an inconsistent group where typical and flat patterns are seen on alternate days. In the current study, salivary cortisol samples were collected on one day.

The results from this study were similar to the Smyth et al. (1997) study in that there were three groups of cortisol diurnal rhythm found: 1) a typical group of peak in the morning and decline in the afternoon (n = 92, 64.3%), 2) a flat diurnal group (n = 18, 12.5% - less than 0.01µg/dL change from morning to afternoon) and 3) an opposite diurnal rhythm group (n = 33, 23%), meaning that the cortisol was low in the morning and high in the afternoon. Collecting data on a second or third day may have shed light on the group that had an opposite diurnal rhythm. It would be helpful to see if these levels were consistent or if they changed depending on the day they were collected. Unfortunately, this type of collection in a school setting was not feasible financially, and the disruption to the academic day would not be acceptable to the administration.

The normal diurnal rhythm was an important aspect in this study because when an adolescent does not have a normal rhythm, there may be different implications associated. The results of the current study were comparable to those in the pilot study: normal diurnal rhythm (70%), flat cortisol rhythm (9.6 %), and opposite diurnal rhythm
(19.3%). Out of the 17 adolescents who had flat diurnal rhythms, 77.8% \((n = 13)\) had were in late-to post-pubertal status.

Van den Bergh et al. (2008) found that adolescents who had a flat diurnal rhythm also exhibited greater emotional distress. In the current study, out of the 18 adolescents who had a flat diurnal rhythm, 38.9% \((n = 7)\) that also had clinically significant depressive symptoms \((\text{CESD}-10 > 9)\). Doane et al. (2012) found that adolescents with past episodes of depressive symptoms had flatter cortisol slopes \((r = 0.14, p < 0.05)\). Perhaps those adolescents who did not self-report depressive symptoms were withholding information or may not be having depressive symptoms currently but have had problems with depressive symptoms in the past. The current study did not ask about previous depressive symptoms, only if the adolescent had a current depression diagnosis or was on medications for depression. In future studies, inquiring about previous depressive symptoms or conditions may be important to consider.

As the adolescents were finishing their 2\textsuperscript{nd} data collection period in the afternoon, they were asked a question (“Has anything stressful occurred today?”) to help understand any other stressor that may have occurred on the day of data collection. This information was collected to help explain unusual diurnal rhythms. Answers ranged from issues such as fighting with mom or dad, breaking up with a romantic partner, failing a test, taking an exam, homecoming, or being left out of a friendship activity, among several other answers. Out of those adolescents who had opposite diurnal rhythm scores \((n = 34)\), there were 17 (50\%) who self-reported a stressful event had occurred that day.

Oldehinkel and Bouma (2011) discuss that it is unclear whether depressive symptoms lead to stress that can change cortisol levels, and suggests that if the increase
in stress changes the cortisol levels, this may lead to depressive symptoms. For this study, those adolescents who had scores above 9 on the CESD-10 or clinically significant depressive symptoms also had significantly higher perceived stress scores. When looking at the stressful life events with regard to depressive symptoms, those adolescents who scored above a 9 on depressive symptoms also had significantly higher stressful life event scores and life change units. This study provides useful information; yet, there is room for more research on the HPA axis variation in adolescents. In a future study, the ideal scenario would be collecting at least four or five cortisol samples per day for a period of several days throughout the week. According to Clements (2013), “the gold standard for measuring baseline cortisol levels is to collect at least 50 µL of saliva at wake time, 30 min post, at least one time in the late afternoon, and at bedtime, commonly at 11pm” (p. 216). Furthermore, Clements (2013) stresses that this routine of data collection of cortisol should be done for at least 2 days to account for any systematic affect that could have occurred on a single day. Clements (2013) further highlights that longitudinal studies comparing cortisol levels over a period of time can address issues of within-subject stability, assess how individuals respond to treatment, or account for changes in development.

Kirschbaum and Hellhammer (1989) discuss a known fact that the HPA axis responds sensitively to external stimulation. Such events described above that can happen in the daily life of a 9th grader would produce an expected rise in cortisol. Although this study was not designed to have a “stressor” stimulation during the day of data collection, normal life situations contain various levels of stressors, and depending on the
adolescent’s previous experience with these individual daily stressors, cortisol levels may be different for each individual (Kirschbaum & Hellhammer, 1989).

Although cortisol AM, PM, or cortisol diurnal rhythm did not correlate with CESD-10 or any of the independent variables, there are findings from other studies that support the relationship of cortisol diurnal rhythm with depressive symptoms (Vaillancourt et al., 2008, 2011; Karatsoreos & McEwen, 2009). In the current study, when examining gender and cortisol levels, there were significant differences between the morning cortisol levels between males and females. Females ($\bar{x} = 0.22$, $SD = 0.14$) had higher cortisol than males ($\bar{x} = 0.15$, $SD = 0.88$) in the morning only. These results are also seen in the literature (Rosmalen et al., 2005). The majority of adolescents in the current study (64%) had a normal cortisol diurnal rhythm; with smaller percentages (12.5%) having a flattened rhythm (less than 0.01µg/dL change from morning to afternoon), and 23% having an opposite diurnal rhythm (lower in the morning and higher in the afternoon).

Vaillancourt et al. (2008) studied 12 year old adolescents ($n = 154$) to determine if there was a relationship between bullying and the HPA axis by measuring salivary cortisol in adolescents’ home settings at 20 minutes after awakening, and at 9pm on a Monday, Thursday, and Saturday. Other data collected included a bullying survey, a depression and anxiety survey, and a pubertal status survey. Results indicated that there was very little variation between individuals at different time points, but there was variation (within individuals) between the patterns of cortisol when looking at the different collection days. There were significant differences found between the morning and afternoon cortisol levels where cortisol was higher in the morning than in the
afternoon. A significant relationship was found between cortisol and verbal victimization with a lower level of cortisol found in females who were verbally bullied after controlling for pubertal status, age, depressive symptoms and anxiety. In boys with occasional bullying, the cortisol levels were higher. Over all the examinations of cortisol as compared to the different levels of peer victimization, there was a consistent pattern of hyposecretion of cortisol. Similar findings seen in the current study were that males experienced more physical bullying than females, and females had higher cortisol levels than males. Other researchers have also found these results when comparing males and females when examining cortisol levels (Klimes-Dougan et al., 2001; Netherton et al., 2004)

Vaillancourt et al. (2011) examined 12 year old adolescents \( n = 168 \) with the goal of determining predictive relationships between peer victimization/bullying, depressive symptoms, and salivary cortisol on memory at four different time points. Results indicated that peer victimization, depressive symptoms and cortisol were stable over each time period (6 months apart for Time 1, 2, 3 and Time 4 was 2 to 4 months later). Depressive symptoms and peer victimization were linked at each time period, and peer victimization predicted higher scores of depressive symptoms. This finding was similar to the current study. Another finding of the Vaillancourt et al. (2011) study that was similar to the current study was that cortisol was concurrently not linked to peer victimization or to depressive symptoms. However, depressive symptoms predicted lower morning and evening cortisol at the third time point. As discussed by Vaillancourt et al. (2011), cortisol link to depressive symptoms has been inconsistent in most studies as seen in a meta-analysis by Lopez-Duran et al. (2009).
Besides these two studies from Vaillancourt et al. (2008, 2011), there have been no studies that specifically examined bullying, cortisol, and depressive symptoms by collecting cortisol on routine days of the week. There are other studies found in the literature where researchers collected cortisol and compared this to the relationship with bullying and depressive symptoms; however, all of these studies included a stress response or simulated stress event as part of the study (Knack et al., 2011; Ouellet-Morin et al., 2011; Rudolph et al., 2011; Stroud et al., 2009).

Theoretical and Conceptual Framework Application

McEwen’s Allostatic Load Theory (McEwen, 1998) served as the theoretical framework for the current study. McEwen’s Allostatic Load Theory is centered on the connection between various types and levels of stress and the physiological and psychological consequences that result. The conceptual framework for this study, integrating McEwen’s model, sought to test the relationships among the concepts of stressful life events, life change units, perceived stress, bullying, cortisol, and depressive symptoms with the covariates of gender, race, gender identity, socioeconomic status and pubertal status. In addition, the physiological biomarker, cortisol diurnal rhythm, was examined as a mediator between stressful life events, life change units, perceived stress, bullying, and depressive symptoms, while holding the covariates of gender, race, gender identity, socioeconomic status, and pubertal status constant.

McEwen and Ginaros (2011) discuss that an individuals’ brain is the organ that experiences and adapts to stress. The brain recognizes when there is a potential threat, and then the process of physiological and behavioral responses begin. McEwen (1998)
goes on to say that when an individual is under too chronic stress, this can cause
dysregulation among the mediators of stress, one of which is cortisol. In turn, this
dysregulation results in a change that is called allostatic load and overload. McEwen and
Wingfield (2010) posit that an individual experiences allostatic overload during
prolonged stress when there are not adequate coping resources. At this time the
dysregulation physiologically may be the beginning of disease processes such as
depression or other physiological illnesses such as diabetes, or heart disease.

Stress, as described by McEwen and Akil (2011) can be linked to genetic
changes, and the stress of the social environment can “get under your skin” (McEwen,
2012, p. 17180). The adolescent brain is particularly vulnerable to stress (Romero &
McEwen, 2006). Other investigators examined the impact of stress in a social
environment (Seeman & McEwen, 1996), socioeconomic and social disadvantage effects
of stress in an adolescent (Goodman, McEwen, Dolan, Schafer-Kalkhoof & Adler, 2005),
stress throughout the life-span including development and puberty (Juster et al., 2011),
resilience and adaptation (Karatoreos & McEwen, 2013), and depression (Brown,

When examining McEwen’s Allostatic Load Model, allostatics is affected by three
main variables outside of the individual: Environmental Stressors (work, home,
neighborhood), Major Life Events, and Trauma and Abuse. According to McEwen,
1998, each person has individual differences that contribute to their life trajectory
(genetics, development, and experience). Each of these differences interacting with the
three major variables outside of the individual (Environmental Stressors [work, home,
neighborhood], Major Life Events, Trauma and Abuse) will determine that particular individual’s perception of stress.

In support of McEwen’s model and the conceptual model developed for the current study, results found were supportive of the relationships among stressful life events (SLE, LCU), perceived stress (PSS-10), and bullying (PECK). Additionally, adolescents who reported high stress (SLE, LCU and PSS-10), reported more bullying (PECK), thus perceiving bullying as a threat. The adolescent could become vigilant towards the stress/threat, or he/she may be rendered helpless in the perception of stress.

Depending on how the individual perceives the stress, there may be different behavioral responses to that stress (fight or flight, and personal behaviors such as overeating, smoking, drinking, exercise). This process of evaluating and dealing with the individual stressor culminates in a physiological response involving many chemical mediators that originate in the brain, one of which is glucocorticoids or cortisol (Chrousos & Gold, 1992). The process of allostasis is the body’s way of stabilizing itself in response to the individual differences, the perception of stress, and the behavioral response (McEwen, 2012). The body and brain will either adapt to the stressor or move into allostatic load, where all of the above variables begin to wear down the individual’s ability to adapt to the stressors. If adaptation does not occur, the individual may experience allostatic overload, which is the beginning of the disease state.

Research Question 3 and its hypothesis provide support to McEwen’s Allostatic Load theory by the unique amount of variance that the independent variables of PSS-10 and PECK played in depressive symptoms. The fact that gender identity was the only covariate that significantly contributed to the variance in depressive symptoms fits well
with McEwen’s Allostatic load theory as McEwen (1998) discusses individual differences such as genes, development, and experiences all contributing to the physiologic response to stress as well as allostatic load or overload, which in the case of the current study would be looked upon as depressive symptoms that occur related to high levels and perception of stress (McEwen, 2005; McEwen, 2012).

Research Questions 4, 5 and 6 and their hypotheses failed to be proven in the current study due to the non-significant relationships found between cortisol and the independent variables as well as the dependent variable. However, when cortisol diurnal rhythm was evaluated by categories of normal diurnal rhythm, flattened diurnal rhythm, and opposite diurnal rhythm, there were signs of dysregulated cortisol, which McEwen and Seeman (1999) speak of when the stress response fails to shut down even after the stressor has ended. The flattened diurnal rhythm (change of less than 0.01 µg/dL from morning to afternoon in cortisol levels) and the opposite diurnal rhythm (cortisol increasing from morning to afternoon instead of the expected pattern of decreasing from morning to afternoon) would indicate dysregulation or could indicate variation between the individual on any given day.

The literature supports that a high flattened rhythm is seen in individuals experiencing depressive symptoms (Klimes-Dougan et al., 2001). A low flattened or “blunted” diurnal rhythm has been seen in individuals who have been exposed to trauma at an early age (Bevans et al., 2008; Shirtcliff et al., 2011). Even though the morning and afternoon cortisol levels were still within the normal range for individuals, those adolescents who only had 0.01 µg/dL change from morning to afternoon (n = 18) appear to be “low and blunted” when looking at the cortisol diurnal rhythm.
When examining the adolescents who had the opposite diurnal rhythm (increase from morning to afternoon), the cortisol levels’ increase from morning to afternoon ranged from \(0.02 \mu g/dL-0.53 \mu g/dL\). This slight increase in cortisol, thus opposite cortisol diurnal rhythm could have been related to timing of the saliva collection (shorter time between collections), stressors that occurred on the day of data collection, or an individual variation among adolescents “basal or trait” cortisol diurnal rhythm (Shirtcliff & Essex, 2008).

When examining cortisol diurnal rhythm and basal cortisol levels in a group of adolescents, Shirtcliff and Essex (2008) found that basal cortisol levels accounted for 20.8% of the variability of cortisol levels. These researchers also found significant day to day variability. When Shirtcliff and Essex (2008) collected cortisol diurnal rhythm levels over several days, some adolescents had higher cortisol levels and a steeper slope on the first day of data collection as compared to other days. This finding suggests that there may be novelty that influences the saliva collection.

Shirtcliff and Essex (2008) found that adolescent males had lower basal cortisol and flatter diurnal rhythms than females. The same was true in the current study; there were eleven males who had a flattened diurnal rhythm and seven females with a flattened diurnal rhythm. More females had a normal diurnal rhythm as compared to males (females, \(n = 66\) or 71.7%, males, \(n = 26\) or 28.3%). These were significant differences in diurnal rhythm patterns \(\chi^2 = 7.296, df = 2, p = 0.026\). These findings further support McEwen’s Allostatic load model in that there are individual differences from day to day and in the way each person adjusts to stressors.
Limitations

In this study there were research design and method limitations that must be acknowledged. The first limitation is a cross-sectional data collection that took place at one time during the 9th graders school year in two small geographic locations. A cross-sectional design provides only a brief view of the variables. A longitudinal study would provide a better view of how these variables may change over time for a 9th grade adolescent. Adolescence is a period of biological, developmental, psychosocial, and emotional changes, and there is known individual variation in the timing of these changes (Cushman, 2006; Dahl & Gunnar, 2009; Friedlander et al., 2007).

Longitudinal studies are possible to do in a school setting but may not be as feasible as a cross-sectional design. Feasibility issues such as adolescents who may move out of the school district, or may be unwilling to cooperate with a second data collection, and additional expenses in maintaining contact with participants over time may make longitudinal studies in a school setting not possible (Brenning, Soenens, Braet, & Beyers, 2013; Cornell et al., 2013; Ryoo et al., 2014; Weigel et al, 1998). In addition to the above limitations with a cross-sectional design, no causal inferences can be made when examining the study variables and the outcome of depressive symptoms. Polit and Beck (2012) discuss that in order to establish cause and effect, there must be a temporal and empirical relationship between the variables. A randomized clinical trial would provide better control over confounding variables so that causal inferences could be made. The current study was a non-experimental study and only establishes that there was a relationship between the variables.
Self-report is also a study limitation that must be recognized. Adolescents in a school computer room setting may have difficulty with recall of stressful life events that have occurred in the last year. Depending on the day of the week, stressful events that occurred before or during school, the presence of other adolescents in the room, or the possibility that an adolescent does not want to reveal sensitive information to the researcher must also be considered (Kissling, 1996; Sawyer et al., 2008; Tourangeau & Yan, 2007). As also seen in the Berlan et al. (2010) study, there may be adolescents in the current study who underestimated any bullying experiences, particularly with relational aggression. Moreover, it may be difficult to remember the actual experiences of bullying depending on when they occurred.

Adolescents are often reluctant to self-report smoking. The small numbers of adolescents who self-reported as smokers (small cell sizes) must be viewed cautiously. Clements (2013) also discusses substances that affect the cortisol levels such as nicotine caffeine, alcohol, food (Kudielka et al., 2009), and other drugs such as steroids (Granger, et al, 2009). Although information was collected in the first data collection session about whether the adolescent was a smoker, the last time the adolescent smoked, and the last time food was consumed, this information was not collected again in the afternoon session. There was no way to control the lunch periods during a school day, and many of the study adolescents may have just eaten lunch or a snack just before collecting their second cortisol level. The issues of smoking and food could cause subtle changes in cortisol levels; however, collecting data in a school environment is difficult to control.

Threats to internal validity, that is, history or events that took place outside the school setting may have impacted the study findings. For example, the week prior to the
first data collection at School A, a senior student was killed in a car accident. All students in the school were allowed to miss school for the funeral, and counselors were brought into the school to assist with grief from the loss of this well-loved senior student. During the last data collection at School A, there was a sports competition for the State Championship taking place. In that same school, the day after data collection was the beginning of Thanksgiving break. Another event occurring in School A was the Homecoming dance and football game, each of which could be considered stressful, depending on the amount of an adolescent’s involvement in such events. School B had no significant outside-of-school issues that were known to the investigator, but it cannot be confirmed that issues did not occur that could have affected the stress levels of adolescents in the school setting.

Similar to the Cornell et al. (2012) study that only included 9th graders when examining prevalence of bullying and teasing and high school dropout rates, the current study was limited by not including all grades of high school to compare stress, bullying and depressive symptoms; however, including a sample from each grade level was not feasible for this dissertation study. Rice (2012) discusses the importance of examining stressful life events in diverse backgrounds. In the current study, the demographics were representative of the community and entire high school populations; therefore, increasing the diversity of the sample would have been difficult without going to a different school district with a more diverse community demographic make-up.

Conducting biobehavioral research in a school setting is feasible; however as discussed by Clements (2013), measuring cortisol is similar to “aiming a weapon at a moving target” (p. 207). There is not only a peak shortly after awakening, but pulses of
cortisol are released throughout the day (McMaster et al., 2011). The cortisol concentration could be at varying points when the sampling occurs (peak, just after a pulse, just before a pulse, or a pulse in response to a stressor). In addition, the cortisol could be a function of that particular individual (Clements, 2013; Shirtcliff et al., 2011). Therefore, the time of day and moment to moment pulses could cause inaccuracy of study conclusions.

It is clear that single samples of cortisol are not adequate when measuring typical cortisol levels for an individual, and ideally, the cortisol should be measured several times throughout the day and evening on two or more days to obtain high quality results (Clements, 2013). According to Hellhammer and colleagues (2007), a typical design for sampling of cortisol should be upon awakening, 30 minutes after awakening, around noon, in the later afternoon, and just prior to getting into bed. Care was taken to achieve as much consistency as possible; however, there were broad differences in the times of collection between the morning and afternoon cortisol ($\bar{x} = 4$ hrs., 40 min, $SD = 1$ hr., 9 min., range = 1 hr., 40 min. to 6 hrs, 52 min.) due to the nature of the school setting and the requirement of collecting the samples during a non-academic classroom period. These time differences in between samples and the time differences while actually collecting the sample (Morning - $\bar{x} = 1$ to 12 min, $SD = 3$ min., Afternoon - $\bar{x} = 1$ to 14 min., $SD = 3$ min.) could have contributed to some of the unusual diurnal rhythm patterns seen in the cortisol analysis.

While other studies support the relationship between higher cortisol levels in adolescents with depressive symptoms (Lopez et al., 2009; Van den Berg & Van Calster, 2009), these relationships were not supported in this study. It is known that menstrual
cycle phase, oral contraceptives, breast feeding, and pregnancy can affect the levels of cortisol (Kudielka et al., 2009). The current study asked screening questions about oral contraceptives, and pregnancy, but did not inquire about menstrual cycle phase (only age of starting menses) or breast feeding. The majority of females ($n = 92, 98\%$) in this study had begun their menses. Adding a question about the menstrual cycle phase may have been helpful information to obtain; although, retrieving this information may be difficult to collect due to the adolescent age and feelings about discussing their menstrual cycles in a school based study (Kissling, 1996).

One final area discussed by Clements (2013) is that of life style, health, and genetics. Adolescents in the current study were screened with a question about Cushing’s disease and Addison’s disease, both of which can dramatically alter cortisol levels (Gold, Goodwin, & Chrousos, 1988 a, b). However, other physical disorders such as asthma and hypertension could also contribute to changes in cortisol (Chida & Steptoe, 2009).

Several adolescents reported having asthma during the screening, though they stated they were not taking a steroidal medication (exclusion criteria, as this medication impacts cortisol), and were allowed to participate in the study. In the current study, the adolescents were not asked about the age that puberty began, but the females were asked at which age their menses began. Adding a more specific question about the starting of pubertal changes to future studies may help to identify those adolescents who mature earlier as compared to those who mature later (Shirtcliff, 2011).

Exercise in the school setting is also something that is difficult to control when collecting a biobehavioral measure such as cortisol. Evidence indicates a rise in cortisol after intense exercise (Paccotti et al., 2005), but not necessarily after with lower intensity
exercise (Kudielka et al, 2009). Physical education was a regular part of a school day for almost all adolescents who participated in the current study. Some of these adolescents were in physical education just before coming to collect cortisol samples. The level or intensity of their exercise routine is unknown as to exactly what type of exercise occurred on the day of data collection.

In summary, while there are many limitations when collecting data in a school setting, the location and availability of adolescents who are interested in participating cannot be discounted (Tuckman & Harper, 2012). It would be practically impossible to consider all of the above issues that could affect cortisol, or that could affect answers provided for an online computer survey from an adolescent in the 9th grade. Each study done in a school setting will be unique to that particular school. The more consistency that can be achieved in each location with regard to time, activities, and hours of involvement of data collection, the better the results will likely be (Hazler, et al., 2006). As Clements (2013) stated “There should not be discouragement that cortisol has not behaved neatly by rules in research to date” (p. 215). The study of cortisol has provided information with regard to developmental research.

Implications for Professional Practice, Policy, and Future Research

Depressive symptoms are a significant problem to identify in adolescents living in the United States. The National Alliance on Mental Illness (2010-2015) reports that approximately 8% of adolescents meet criteria for major depression on any given day. In fact, one out of five adolescents has experienced depression at some point during this developmental transitional period, with primary care physicians reporting rates as high as
28% for adolescents. One in ten Americans describes at least one episode of depression in their lifetime (CDC, 2012). Depressive symptoms often present in childhood or adolescence (Abela & Hankin, 2008; Kessler, Avenevoli & Merikangas, 2001).

Avenevoli, Swendsen, He, Burstein and Merikangas (2015) presented results from the National Comorbidity Survey-Adolescent Supplement confirming the importance of early identification of depressive symptoms due to the substantial increase in depression in adolescents aged 13 to 18 years. Furthermore, there is immediate concern that more than 25% of depressed adolescents had high levels of distress and impairment (Avenevoli, et al, 2015). The highest expenditure for care and treatment in 2011 for children and adolescents was found to be related to mental health disorders (Agency for Healthcare Research and Quality, 2014). The current study focused on suburban adolescents aged 14 to 16 who are beginning 9th grade, a transitional year in which adolescents are more likely to experience risks associated with biological, developmental, emotional, social, and psychological changes (Bohnert et al, 2013; Ellerbrock & Kiefer, 2013; Hankin et al., 2010; Hauser et al, 2010).

Adolescents in this study reported high levels of stress. Over one-third (39%) of study participants reported depressive symptoms that were clinically significant (CESD-10 score > 9). Rates of stress were also high with regard to stressful life events (SLE), the impact or life change units (LCU) associated with the event, and the perception of stress (PSS-10) identified by the adolescent. The SLE scores were higher than average at 14 stressful life events, and the magnitude of these events weighing an average LCU score of as compared to the normative high LCU score of 170 over the last 12 months. The perceived stress scores were also high as compared to other studies (Lemma et al., 2012;
Sitek, Zadzinska & Rosset, 2012) with an average PSS-10 score of 19 out of a total score of 40 or approximately 87% of study adolescents scoring over 10 out of 40. The current study provides evidence that 9th grade can be a stressful year depending on adolescents’ perception as well as the number and magnitude of stressful events occurring.

*Implications for Professional Practice*

This study population had high rates of depressive symptoms and stress, thus indicating the need for screening, recognition, and early identification of this highly prevalent mental health issue before further mental health decline or disease occurs. Stressful life events and the magnitude of those events as far as social readjustment (life change units) are important to recognize especially when working one on one with an adolescent. Many nurses will interact with adolescents who are experiencing multiple stressful life events on a daily basis (Pavletic, 2011; Young, 2012); therefore, using the CLES-A may prove to be valuable in a school nurse’s or a school counselor’s office.

In the school setting, Allison, Nativio, Mitchell, and Yuhasz (2014) screened adolescents \((n = 182)\) for depressive symptoms using the Patient Health Questionnaire-9 item (PHQ-9) with 11.5% \((n = 21)\) having a positive screen. As a result of positive screens, eight these adolescents were diagnosed with depression and one adolescent having suicidal ideation was involuntarily placed in a psychiatric hospital. Other adolescents were referred to a multidisciplinary school-based Student Assistance Program team for further evaluation.

In the primary care setting, Taliaferro et al. (2013) identified Primary Care Providers (PCPs) \((n = 537)\) and collected data using an online survey to determine
identification and management practices of depression in adolescents. Both physicians (family practice and pediatricians) and pediatric nurse practitioners provided information that they felt most prepared them to refer adolescents for mental health services. Pediatric clinicians reported more familiarity with depression screening, yet they were less likely to administer a written depression screening instrument. Most PCPs reported only screening high-risk patients that had warning signs or screening only in response to specific complaints.

Providing routine screening for depressive symptoms in a school setting or other locations where adolescents frequent may be an appropriate step in recognizing depressive symptoms before they progress further into major clinical depression and affect the trajectory of the adolescent’s life. The high CESD-10 scores in the current study confirm that depressive symptoms are worth evaluating in this vulnerable population as there is sufficient empirical evidence to show that depressive symptoms in adolescence may lead to depression in an adult population (Braet et al., 2012; Chen et al., 2011; Georgiades et al., 2006).

**Implications for Policy**

This study’s finding of 39.2% of adolescents having clinically significant depressive symptoms affirms The U.S. Preventative Services Task Force’s (2014) recent report to the U. S. Congress that recognized depression screening as a high priority for adolescents. This task force recommends screening adolescents aged 12 to 18 years in the primary care setting using the Patient Health Questionnaire-9 (PHQ-9) or the Beck Depression Inventory. Benefits of screening are earlier detection and intervention (Allison et al., 2014). Along with the benefits of screening, harms of screening must be
also discussed. When adolescents are screened, it is important to have a plan in place as to referral to counselors or psychiatric professionals (Husky et al. 2011). Reynolds (1986) discusses a procedure with three stages in a school setting: 1) School-wide assessment using a self-report instrument for depressive symptoms. 2) Repeat assessment of any adolescent who scored clinically significant symptoms on the first self-assessment, 3) Individual clinical interviews for adolescents who score clinically significant depressive symptoms on the 2nd self-assessment. Although screening adolescents for depressive symptoms is important, screening alone may bring to light that the adolescent has thoughts suicidal ideation or is considering acts to prepare for suicide. These issues are important to monitor closely while the adolescent is receiving psychotherapy or on selective serotonin reuptake inhibitors (Williams, O’Connor, Eder, & Whitlock, 2009).

While the U. S. Preventative Services Task Force is proposing that routine screening for depressive symptoms in adolescent populations occur in primary care settings, the current study provides evidence to show that screening for depressive symptoms is feasible in a school setting and may capture data that could identify more students in a high school setting who are suffering in silence. Penden (2005) concurs with the importance of identifying depressive symptoms in adolescents. In addition, providing interventions for coping and improved family functioning is suggested. It is likely that most mental health problems first occur during the transition period of adolescence (Merikangas et al., 2010). Therefore, screening and recognition of depressive symptoms at a time when they are known to occur may provide a path to a higher quality of life with possible intervention for depressive symptoms before they progress into a diagnosis of clinical depression.
Bright Futures has been recognized as the standard for preventative health insurance coverage for children and adolescents by the Patient Protection and Affordable Care Act (American Academy of Pediatrics [AAP], 2015). These standards highlight key areas related to mental health that is suggested to discuss during a primary care office visit, and these topics include but are not limited to: stability of moods, depression, suicidal ideation, peer relationships, transition to high school, family conflict and sexual identity (AAP, 2002). The Pediatric Symptom Checklist, a screening tool designed to identify emotional, behavioral, and cognitive problems in children and adolescents, is recommended to be used in primary care so that early intervention can occur.

Healthy People 2020 have a goal of reducing the proportion of persons who experience a major depressive episode and increasing the proportion of children and adolescents who receive treatment for mental health disorders (Healthy People.gov, 2012). The current study’s combined findings of not only clinically significant depressive symptoms in 39.2% of adolescents but also finding that approximately 80% of adolescents scored higher on the Coddington Life Event Scale for Adolescents than the normative high score, and 83.9% of adolescents scored in the middle range of Perceived Stress Scale - 10 provides justification for the Healthy People 2020 goal.

Implications for Future Research

*Biobehavioral Research*

The feasibility of collecting saliva in a school setting may be what drives the future of studies with groups of children or adolescents when evaluating for mental health disorders (Granger, 2012). Currently there are many different types of screening tools to
measure depressive symptoms, but there is no recognized biological marker to add substance to the screening tools (Owens et al., 2014). While there was no direct correlation between cortisol and depressive symptoms, the recognition of abnormal diurnal rhythms is an important finding.

Patricia Grady (2011), Director of The National Institute of Nursing Research stresses the importance of including integration of biological and behavioral sciences as a priority to improving the physical and mental health of individuals in the strategic plan for the future of nursing research. Granger et al., (2012) posits that salivary samples may be used to measure cortisol as well as many other types of physiological markers as a non-invasive, cost-effective way to document changes in stress-related physiology.

Biomarkers beyond Cortisol. Although the mediation hypothesis of cortisol diurnal rhythm did not prove to be supported in the current study, other researchers contend that cortisol is seen at both high and low levels in adolescents who have depressive symptoms (Owens, et al., 2014). There is mounting evidence that other biomarkers may provide information about the pathological beginnings of depression. Miller and Cole (2012) studied inflammatory markers in female adolescents (n = 147) aged 15 to 19. Approximately 28% of adolescents in their study had a depressive episode. During these episodes of depressive symptoms, these adolescents had higher circulating IL-6 as compared to visits when they were not experiencing depressive symptoms. Similar patterns were seen with levels of C-reactive protein. Other researchers have examined testosterone, DHEA, and cortisol to determine if these hormones predict negative emotionality, finding that hormones that change during puberty may be an
important key to understanding adolescent moods (Marceau, Dorn, & Susman, 2012; Marceau et al. 2014; Matchock, Dorn, & Susman, 2007).

Other Risk Factors Related to Depressive Symptoms

Substance Abuse. Although there were very few self-reported smokers in the current study, areas to consider for future research would be examining the use of alcohol, drugs, and the frequency of smoking among adolescents and how this relates to those who have depressive symptoms. Pang et al. (2014) studied adolescents ($n = 476$, mean age of 14.5 years) using self-report surveys and the CESD-20 to examine depressive symptoms and substance use initiation. Results found were that depressive symptoms were associated with a lifetime use of substances such as: cigarettes, tobacco, marijuana, alcohol, inhalants, and pain medications. Pang et al. (2014) conclude that targeting adolescents by screening for depressive symptoms early would be important in preventing future abuse issues.

Family History. Previous emotional problems, family history of depression, or mental illness could also contribute to an understanding of current depressive symptoms in adolescents. These questions were not explored in the current study. Swartz, Williamson and Hariri (2015) discuss that there is a gap of knowledge related to family history of depression and threat-related amygdala reactivity. The amygdala is the emotional center of the brain and is responsible for detection of fear and preparation for a threat (Morrison & Salzman, 2010). Understanding the family history of depression and determining how an adolescent responds to stressful life events may serve as a premorbid biomarker of risk of developing internalizing disorders or depression (Swartz et al., 2015).
**Settings.** A future study could include a comparison between different types of schools such as urban, suburban, and rural schools or religious/private schools to see if there are differences among these populations of adolescents with regard to depressive symptoms. Examining participation in extracurricular activities, enrollment in advanced or International Baccalaurean classes or involvement in school groups may also contribute more to the literature in helping to understand the amount of stress that occurs in this age group.

Conducting a longitudinal study (9th through 12th grade) in the future could also provide information to determine if stress, bullying, and depressive symptoms continue as the adolescent progresses through high school. Furthermore, examining this path may help to determine if the adolescent in 9th grade continues school until graduation.

**Measures**

The PSS-10, the PECK survey, and the CESD-10 were feasible to use in a school setting with this 9th grade population in terms of length of time it took to take these surveys in an online format. The pubertal status scale was an effective measure of pubertal status; however, surveying more adolescents may be necessary in the future to increase the reliability of the different gender components of this measure.

The CLES-A was developed to determine how psychological trauma is related to the etiology of both physical and mental disease. When interpreting the CLES-A, issues that need to be considered include contextual factors such as genetic predispositions of the adolescents or environmental characteristics of the participant’s home. Data on these contextual factors were not collected for the current study. The results of the CLES-A could be used by a clinician to more carefully identify problems in an adolescent and to
develop intervention tailored for specific problems. Other information that could also be integrated with the CLES-A might include family and medical history, school records, and background of the adolescent’s growth and development (Coddington, 1999). All of this data would be difficult to collect in a school setting such as the one used for this current study, but could possibly be collected in a clinic-type setting. The CLES-A is only one assessment and should be used as a screen to alert those working with adolescents that further investigation may be warranted (Coddington, 1999). Since this was a research study and not a diagnostic investigation, information from the CLES-A was not provided to anyone at the school setting.

Garrison et al. (1987) also measured the adolescent living arrangement and found that adolescents who lived with both parents had the lowest number of stressful life events (SLE = 21.96) as compared to those who living with only the father (SLE = 28.91). The information collected on living arrangement in the current study was not part of the research questions; however, this information may prove to be helpful for future studies that look more closely at depressive symptoms and the home environment.

Summary

A detailed discussion of the study findings in relation to the literature on stress, bullying, cortisol, and depressive symptoms in 9th grade adolescents has been presented in this chapter. As seen in the literature above, this study finds that 9th grade is a particularly stressful experience for some adolescents. It is important to recognize this vulnerable population to identify those adolescents who are under a great deal of stress and those adolescents with depressive symptoms.
In this study, the majority of adolescents were White, with approximately twice the amount of females as males. Participation rates were excellent at 55%. Stress levels were high as measured by stressful life events, life change units, and perceived stress. Other studies have measured stress with similar instruments, and the results of the current study appear to be higher in each of the stress related variables. Bullying scores were relatively low in comparison to other U. S. National studies; however, the rate of bullying was similar to the study that was used for the instrument development. Verbal/relational bullying appears to be the most common among the types of bullying endorsed in this study as compared to cyber, physical, and cultural bullying.

Depressive symptoms were predicted from perceived stress, bullying, and gender identity variables using General Linear Models with perceived stress contributing the most variance towards depressive symptoms when controlling for other stress measures and covariates. Cortisol diurnal rhythm did not mediate the relationship between the independent variables and the dependent variable. However, the results of cortisol diurnal rhythm were similar to those seen in other studies discussed in this chapter. The measurement of a salivary biomarker was feasible in a school setting, although this procedure was not standardized because of the complications of working with students while taking “elective classes” and the times being out of the researchers control. Thus, the results must be viewed with caution in light of the length of time between the morning and afternoon samples.

This research was representative of McEwen’s Allostatic Load Theory in that the more stress adolescents experience, the more depressive symptoms adolescents may face. Although, this study was not diagnostic of clinical depression, evidence from the
literature discussed in this chapter indicates that on any given day, adolescents may experience clinically significant depressive symptoms. This finding was certainly true in the current study.

National policy is already in progress with regard to depression screening recommendations in a primary care setting for adolescents; yet, this study shows that it is feasible to screen for depressive symptoms in a school setting. If screening for depressive symptoms takes place in the school setting, there must be a plan in place to assist those adolescents identified with clinically significant symptoms. Nurses, counselors, and other mental health professionals working in the school setting may be key individuals to conduct this type of screening.

It is clear that more research in bullying is warranted, particularly in the LGBTQ and other populations. Research discussed in this chapter points out that adolescents who are different from their peers with regard to physical looks, sexual minority, or disability are targeted by bullies and are often socially isolated and experience harassment and violence from peers. Taking steps to screen, monitor, and refer adolescents who are transitioning to 9th grade may increase quality of life and overall mental health well-being, thereby making 9th grade and perhaps future high school years a better experience overall.


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APPENDIX A

INSTRUMENTS
Center for Epidemiological Studies Depression Scale -10 (CESD-10)

The next sets of questions are related to how you have felt in the last 30 days.

I was bothered by things that usually don't bother me

never
rarely
sometimes
most days
every day

I have trouble keeping my mind on what I am doing
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I felt depressed
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I felt that everything I did was an effort
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I felt hopeful about the future
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I felt fearful
all of the time (5-7 days)
occasionally or a moderate amount of the time (3-4 days)
sometimes or a little of the time (1-2 days)
none or rarely (less than a day)
My sleep was restless
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occcasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I was happy
all of the time (5-7 days)
occcasionally or a moderate amount of the time (3-4 days)
sometimes or a little of the time (1-2 days)
none or rarely (less than a day)

I felt lonely
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occcasionally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

I could not get going
none or rarely (less than a day)
sometimes or a little of the time (1-2 days)
occcidentally or a moderate amount of the time (3-4 days)
all of the time (5-7 days)

Note. Scale Ranges from a score of 0-30.
Answers above are rated 0, 1, 2, 3 points from first answer to last answer.
This scale was part of the online survey developed through Survey Gizmo.
PERCEIVED STRESS

The next sets of questions are related to your thoughts and feelings during the last month. Please click on the response that describes how often you felt or thought a certain way.

In the last month, how often have you been upset because of something that happened unexpectedly?

never
almost never
sometimes
fairly often
often

In the last month, how often have you felt that you were unable to control important things in your life?

never
almost never
sometimes
fairly often
very often

In the last month, how often have you felt nervous and stressed?

never
almost never
sometimes
fairly often
very often

In the last month, how often have you felt that things were going your way?

very often
fairly often
sometimes
almost never
never

In the last month, how often have you felt confident about your ability to handle personal problems?

very often
fairly often
sometimes
almost never
never
In the last month, how often have you felt that you could not cope with all the things you had to do?
never
almost never
sometimes
fairly often
very often

In the last month, have often have you felt that you were able to control irritations in your life?
very often
fairly often
sometimes
almost never
never

In the last month, how often have you felt that you were on top of things?
very often
fairly often
sometimes
almost never
never

In the last month, how often have you been angered because of things that were outside of your control?
never
almost never
sometimes
fairly often
very often

In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
never
almost never
sometimes
fairly often
very often

Note. Scale ranges from 0-30.
Answers are rated 0, 1, 2, 3, 4 from first answer to last answer.
This scale was part of an online survey on Survey Gizmo.
PERSONAL EXPERIENCES CHECKLIST (PECK)

Thinking about the last month or so of school, how often do the following things happen?

Other kids say mean things behind my back
never
rarely
sometimes
most days
every day

Other kids try to turn my friends against me
never
rarely
sometimes
most days
every day

Other kids tell people not to hang around with me
never
rarely
sometimes
most days
every day

Other kids tease me about things that aren’t true
never
rarely
sometimes
most days
every day

Other kids ignore me on purpose
never
rarely
sometimes
most days
every day
Other kids call me names because I can’t do something
never
rarely
sometimes
most days
every day

Other kids make rude gestures at me
never
rarely
sometimes
most days
every day

Other kids tell people to make fun of me
never
rarely
sometimes
most days
every day

Other kids call me names because I’m a bit different
never
rarely
sometimes
most days
every day

Other kids make fun of my friends
never
rarely
sometimes
most days
every day

Other kids make death stares at me
never
rarely
sometimes
most days
every day
Other kids say nasty things to me by SMS
never
rarely
sometimes
most days
every day

Other kids threaten me over the phone
never
rarely
sometimes
most days
every day

Other kids send me nasty e-mails
never
rarely
sometimes
most days
every day

Other kids harass me over the phone
never
rarely
sometimes
most days
every day

Other kids say nasty things about me on websites
never
rarely
sometimes
most days
every day

Other kids send me computer viruses on purpose
never
rarely
sometimes
most days
every day
Other kids say nasty things about me on an instant messenger or chat room
never
rarely
sometimes
most days
every day

Other kids make prank calls to me
never
rarely
sometimes
most days
every day

Other kids hit me
never
rarely
sometimes
most days
every day

Other kids punch me
never
rarely
sometimes
most days
every day

Other kids kick me
never
rarely
sometimes
most days
every day

Other kids shove me
never
rarely
sometimes
most days
every day
Other kids trip me over
never
rarely
sometimes
most days
every day

Other kids tell people to hit me
never
rarely
sometimes
most days
every day

Other kids say they’ll hurt me if I don’t do things for them
never
rarely
sometimes
most days
every day

Other kids wreck my things
never
rarely
sometimes
most days
every day

Other kids play practical jokes on me
never
rarely
sometimes
most days
every day

Other kids make fun of my language
never
rarely
sometimes
most days
every day
Other kids make fun of my culture
never
rarely
sometimes
most days
every day

Other kids tease me about my voice
never
rarely
sometimes
most days
every day

Other kids won’t talk to me because of where I’m from
never
rarely
sometimes
most days
every day

Note. Scores range from 0-128. This scale was part of an online survey on Survey Gizmo.
Scoring
0 – never
1 – rarely
2 – sometimes
3 – most days
4 – every day
Self-Rating Scale for Pubertal Development

The next sets of questions are related to your physical maturity or pubertal status. Your answers will be confidential and if you do not want to answer any of the questions, you may do so.

Males and Females answer the following questions:

Would you say that your growth in height......
has not begun to spurt
has barely started
is definitely underway
seems complete
I don't know

How about the growth of your body hair?
Would you say that the hair on your body (legs, under arms, pubic area)
has not begun to spurt
has barely started
is definitely underway
seems complete
I don't know

Have you noticed any skin changes? (pimples/acne)
skin has not yet started changing
skin has barely started changing
skin changes are definitely underway
skin changes seem complete
I don't know

Males questions only:

Have you noticed a deepening of your voice?
voice has not yet started changing
voice has barely started changing
voice changes are definitely underway
voice changes seem complete
I don't know

Have you begun to grow hair on your face?
facial hair has not started growing
facial hair has barely started growing
facial hair is definitely started
facial hair growth seems complete
I don't know
Female questions only:

Have you noticed your breasts have begun to grow?  
has not yet started growing  
have barely started growing  
breast growth is definitely underway  
breast growth seems complete  
I don't know

Have you begun to menstruate (started your period)?  
Yes  
No

How old were you when you started your period?  
Answered by typing in the box, the age when period started

Note. For items 104 on the female version and all of the items on the males’ version, response options were: not started yet (1 point); barely started yet (2 points), definitely started (3 points); seems complete (4 points); I don’t know (missing). Yes on the menstruation (4 points); No on menstruation (1 point). Point values are averaged for all items to give a Pubertal Development Scale (PDS) score.

To compute Pubertal Category for males use body hair growth, voice change and facial hair growth as follows:  
Pre-pubertal= 3  
Early-Pubertal = 4 or 5 (no 3 point responses)  
Mid-pubertal = 6, 7, or 8 (no 4 point responses)  
Late-pubertal= 9-11  
Post-pubertal = 12

To compute Pubertal Category Scores for females use body hair growth, breast development and menarche as follows:  
Pre-pubertal = 2 and no menarche  
Early-Puberty = 3 and no menarche  
Mid-puberty = 4 and no menarche  
Late-puberty = 5-7 and menarche  
Post-pubertal = 8 and menarche
Coddington Life Event Survey – Adolescents

Available from publisher, Multi-Health Systems, Inc. (http://www.mhs.com/)
APPENDIX B

RECRUITMENT LETTER TO PARENTS
Dear Parent or Guardian,

Your 9th grader has been invited to participate in a research study at Satsuma High School. The purpose of this study is to examine the effects of stressful life events, perceived stress, cortisol (stress hormone) and bullying on depressive symptoms in adolescents in the 9th grade. I am a doctoral student at the University of Alabama at Birmingham and doing this study as part of my degree requirements.

We are inviting adolescents who are in 9th grade and between 14-16 years old to participate in the study. We will enroll the first 75 adolescents who turn in a completed informed consent document (signed by their parent/guardian). Then these adolescents will meet with the study investigator, Susan G. Williams MSN, RN.

In this information packet (sent home from school) you will find the consent form and a refusal form. The procedures for this study are described within the enclosed informed consent document.

- If you agree to allow your adolescent to participate, please sign the enclosed consent form. Your adolescent must return this consent form signed by a parent or guardian to the school in the large green envelope provided.

- If you choose for your 9th grader not to participate, please have your 9th grader return the packet to the school nurses office with the signed refusal form.

- Please give the large green envelope to your adolescent. Ask them to return the envelope to school and place it in designated box in the high school nurse’s office.

The informed consent document will be reviewed individually with each adolescent interested in participating. Ninth graders will also sign the same consent form at the school on the day prior to the day of data collection.

Any 9th grader involved in this study may choose to stop participating at any time. This will not affect his or her class standing or grades. Please note that once the participating adolescent has completed the entire data collection (including saliva collection in the morning and the afternoon and five questionnaires), the adolescent will receive a $5 gift card from Smoothie King. In addition, any 9th grader who returns the signed consent form or signed refusal form will receive a small incentive (mechanical pencil, pen, and high lighter) regardless if he/she qualifies for the study.

I want to thank you in advance for your time and consideration. Please feel free to contact me with any questions or concerns regarding this study. I may be easily reached at (251) 490-7893. I appreciate the opportunity to work with your adolescent and Satsuma High School.

Sincerely,

Susan G. Williams MSN, RN
Reminder to Student ___________________________________________________

The research study with Susan G. Williams from the University of Alabama at Birmingham will take place on_____________. Please do not eat, smoke or drink anything except water one hour before your time listed below for coming to the computer room________________________. You will be provided with a snack after the morning data collection (____________) and, you will receive a Smoothie King Gift card after the afternoon data collection (____________). Thank you!

Note. Student name, date, location, and times were filled in on individual students.
APPENDIX D

SCREENING FORM
Screening form for 9th grade study

1. Name (print) ____________________________________________

2. Age__________________________________________________

3. Can you read and speak English?__________________________

4. Do you know how to use the computer to take a survey?________

5. Are you pregnant (females only)____________________________

6. Do you have one of the following diagnosis- Clinical depression, BiPolar Disorder, Cushing’s or Addison’s disease?____________________________

7. Are you on any routine medications? If yes, please list________________________

______________________________________________________________________

Note. Screening form was completed with the student in a private office with investigator
APPENDIX E

INSTITUTIONAL REVIEW BOARD APPROVAL LETTER
Form 4: IRB Approval Form  
Identification and Certification of Research  
Projects Involving Human Subjects  

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00065960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator: WILLIAMS, SUSAN G  
Co-Investigator(s): DAVIS, SARA LAUBINGER  
Protocol Number: X130903004  
Protocol Title: Stressful Life Events, Perceived Stress, Cortisol, and Depressive Symptoms of 9th Grade Adolescents  

The IRB reviewed and approved the above named project on 8/25/14. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.  
IRB Approval Date: 8/25/14  
Date IRB Approval Issued: 8-27-14  
IRB Approval No Longer Valid On: 8-25-15

Julie Linn, M.D.  
Acting Chair of the Institutional 
Review Board for Human Use (IRB)

Investigators please note:  
The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.
CONSENT FORM

TITLE OF RESEARCH: Stressful life events, perceived stress, cortisol, bullying and depressive symptoms in a 9th grade adolescent high school population

IRB PROTOCOL: X130903004
INVESTIGATOR: Susan G. Williams MSN, RN;
Anne Turner-Henson PhD, RN, FAAN (UAB Faculty Mentor)
SPONSOR: The University of Alabama at Birmingham School of Nursing

For Children (persons under 19 years of age) participating in this study, the term “You” addresses both the participant (“you”) and the parent or legally authorized representative (“your child”).

Purpose of the Research

We are asking you to take part in a research study. This study is designed to examine the effects of stressful life events, perceived stress, cortisol and bullying on depressive symptoms in adolescents in the 9th grade. The researcher will enroll 150 adolescents in the 9th grade from Saraland High School/Satsuma High School who are capable of completing four surveys on a school computer and one survey about stressful life events with paper and pencil and two additional questions. The first 40 9th grade students who are eligible will be enrolled in the study.

Explanation of Procedures

All data collection will occur on one day at the school, during school hours. All information will be collected during a particular class on one school day. It will take approximately one hour when you first arrive at the school and 30 minutes at the end of the school day. The study activities will be scheduled so that you will not miss class information or school work.

You will be asked to complete four short questionnaires (Perceived stress survey, Depressive symptoms survey, Pubertal status survey, Bullying survey) and demographic information (age, gender, race, gender identity, and school lunch status) for a total of approximately 73 questions, on the school computers. The questionnaires will be completed on secure school computers using a Survey Gizmo, a secure internet site.

You will be asked to provide two samples of saliva (spit) (morning, afternoon). The saliva (spit) will be used to measure your cortisol level (cortisol is a measure of stress). The saliva sample will be destroyed once the pilot study is completed.

You will be asked to arrive at the designated classroom at 7:30 am and will be instructed on how to collect saliva by allowing spit to collect in a small plastic collection tube. This will take approximately 15 minutes including time to rinse the mouth out with water. Saliva samples will be stored on ice and frozen until the data collection is completed. At that time, the saliva samples will be shipped overnight to Salimeters Laboratory. Once the saliva samples are analyzed, they will be discarded. No further use of the saliva samples will take place other than for the cortisol analysis purposes of this study.

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In the afternoon, you will be asked to complete a 50 question survey about stressful life events with paper and pencil and an additional two questions will be asked about any stress occurring during your school day and how has your 9th grade experience been so far?

Risks and Discomforts

You may possibly experience some psychological discomfort or potential embarrassment related to collecting saliva (spit) or during the completion of the questionnaires; however, the risk involved is minimal. Four questionnaires will be completed on a private computer in your school computer laboratory. One questionnaire will be completed on paper and pencil with an additional two questions about your stress during the school day and your 9th grade experience so far this year. If you become upset or anxious during any parts of the data collection procedures, the data collection procedures will be stopped. You can stop answering the questions at any time.

The study investigator will examine the result of the depressive symptoms survey on the day you take the survey. We will look at the depressive symptoms questionnaire you completed, and if your score is high, we will refer you to the school nurse and the counselor.

Benefits

You may not benefit directly from taking part in this study. However, this study may help health professionals in developing better programs for promoting the improvement of mental health in adolescents.

Alternatives

The alternative is for you not to participate in this study.

Confidentiality

Information obtained about you for this study will be kept confidential to the extent allowed by law. The research results may be published for scientific purposes; however, your identity will not be revealed in any way by name. Research information that identifies you may be shared with the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB) and others who are responsible for ensuring compliance with laws and regulations related to research, including the Office for Human Research Protections (OHRP).

The only time your identity would not be confidential is if you show significant distress (crying or upset) after completing the surveys or if you score >9 on the depressive symptoms survey. You will, at that time, be referred to the school nurse and/or counselor.

In order to minimize the risk of a breach in confidentiality, all questionnaires on the computer will be coded with a unique study code number. The study data will be stored on a password-protected, encrypted computer drive and locked in a cabinet that will be accessible only to the principal investigator of this study.
Voluntary Participation and Withdrawal

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your class standing or class grades. If you would like to withdraw, please notify Mrs. Susan G. Williams.

Cost of Participation

There will be no cost to you for taking part in this study.

Payment for Participation in Research

You will receive a $5 gift card to a local food establishment. You will receive the $5 gift card once you have completed all physiological measures (saliva/spit collection) and the five questionnaires.

Questions

If you have any questions, concerns, or complaints about the research please contact Mrs. Susan G. Williams MSN, RN at 251-490-7893. She will be happy to answer any of your questions. You may also contact the supervising faculty mentor associated with this study, Dr. Anne Turner-Henson at (205) 934-7533. If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the office of the IRB (OIRB) at (205) 934-3789 or 1-800-822-8816. If calling the toll-free number, press the option for “all other calls” or for an operator/attendant and ask for extension 4-3789. Regular hours for the OIRB are 8:00am to 5:00 p.m. Central time, Monday through Friday. You may also call this number in the event the research staff cannot be reached or you wish to talk to someone else.

Legal Rights

You are not waiving any of your legal rights by signing this informed consent document.
Signatures

Your signature below indicates you agree to participate in this study. You will receive a copy of this signed consent form.

Name of Participant

Signature of Parent or Guardian  Date

Signature of 9th grade Student  Date

Signature of Principal Investigator  Date
deUniversity of Alabama at Birmingham

AUTHORIZATION FOR USE/DISCLOSURE OF HEALTH INFORMATION FOR RESEARCH

What is the purpose of this form? You are being asked to sign this form so that UAB may use and release your health information for research. Participation in research is voluntary. If you choose to participate in the research, you must sign this form so that your health information may be used for the research.

Participant Name: ___________________________  UAB IRB Protocol Number: 130903004
Research Protocol: Stressful life experiences, perceived stress, cortisol, bullying and Depressive symptoms in 9th grade adolescents  Principal Investigator: Susan G. Williams MSN, RN.
Sponsor: University of Alabama at Birmingham

What health information do the researchers want to use? All medical information and personal identifiers, including past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of whatever kind related to or collected for use in the research protocol.

Why do the researchers want my health information? The researchers want to use your health information as part of the research protocol listed above and described to you in the Informed Consent document.

Who will disclose, use and/or receive my health information? The physicians, nurses and staff working on the research protocol (whether at UAB or elsewhere); other operating units of UAB, HSF, UAB Highlands, Children’s of Alabama, Eye Foundation Hospital and the Jefferson County Department of Public Health, as necessary for their operations; the IRB and its staff; the sponsor of the research and its employees; and outside regulatory agencies, such as the Food and Drug Administration.

How will my health information be protected once it is given to others? Your health information that is given to the study sponsor will remain private to the extent possible, even though the study sponsor is not required to follow the federal privacy laws. However, once your information is given to other organizations that are not required to follow federal privacy laws, we cannot assure that the information will remain protected.

How long will this Authorization last? Your authorization for the uses and disclosures described in this Authorization does not have an expiration date.

Can I cancel the Authorization? You may cancel this Authorization at any time by notifying the Director of the IRB, in writing, referencing the Research Protocol and IRB Protocol Number. If you cancel this Authorization, the study doctor and staff will not use any new health information for research. However, researchers may continue to use the health information that was provided before you cancelled your authorization.

Can I see my health information? You have a right to request to see your health information. However, to ensure the scientific integrity of the research, you will not be able to review the research information until after the research protocol has been completed.

Signature of participant: ___________________________ Date: ________
or participant’s legally authorized representative: ___________________________ Date: ________
Printed Name of participant’s representative: ___________________________
Relationship to the participant: ___________________________

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