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June 29, 2022

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Substance Use and Retrospective Adverse Childhood Experiences: An Ambulatory Assessment of Cortisol Awakening Response

A Plan B Research Project

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA DULUTH

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF ARTS

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June 2022

Abstract

Although support for the relationship between early life stress and substance use in adulthood is well documented, there is a paucity of research examining the relationship between adverse childhood experiences (ACEs), substance use, and cortisol awakening response (CAR) in college-aged adults. Thus, the current studies were designed to address this gap in the literature. The specific aims of this project included determining the relationship between ACE exposure and risky substance use, whether perceived stress mediates the relationship between ACEs and substance use, whether the diurnal cortisol rhythm varies by substance use status and sex, and whether CAR varies by ACE exposure. In Study One, 265 college students completed measures of ACEs, substance use, perceived stress, and mental health. In Study Two, 55 participants self-administered salivary cortisol samples within their place of residence and completed inventories for ACEs, substance use, and mental health. For Study One, perceived stress levels were higher among those with high risk of hazardous drinking, high e-cigarette use, and daily THC/marijuana use. Results also revealed a significant positive relationship between ACEs and drinking consequences and e-cigarette use as well as a mediating role of perceived stress in the relationship between ACEs and drinking consequences. In Study Two, results revealed a medium effect of cortisol collection time point by sex and by risky substance use status. Further, results indicated that those with high ACEs exhibited blunted cortisol levels immediately upon waking compared to those with low ACE exposure. This study contributes to the growing literature base by using a well-established cortisol collection method that has been previously unexplored in the context of ACEs and substance use. The use of self-collected cortisol samples to identify students at risk for hazardous substance use and other health-compromising behaviors has important implications for tailored prevention efforts for those with a history of ACEs.

Keywords: stress, cortisol, adverse childhood experiences, substance use, college students

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Substance Use and Retrospective Adverse Childhood Experiences: An Ambulatory Assessment of Cortisol Awakening Response

Early life stress has been linked to enduring structural and functional alterations in the brain and stress-related neuroendocrine pathways (Lupien et al., 2009). Researchers suggest that these changes may lead to behavioral, affective, and cognitive impairments, as well as diminished psychological and physical health throughout the lifespan (Anda et al., 2006). Support for these associations can be extended from investigations of the effect of early life stress on adulthood physiological outcomes, including stress response, in animal models (for example, see Meaney et al., 1991). Authors of one large study investigated the association between early life stress and behavioral and epidemiological correlates through the Adverse Childhood Experience Study (Felitti et al., 1998; see also Anda et al., 2006). These researchers found that almost two-thirds of the sample reported at least one adverse childhood experience (ACE). The domains of ACEs that were assessed included abuse (physical, emotional, and sexual), neglect (physical and emotional), and household dysfunction (caregiver mental illness, mother treated badly, caregiver divorce or separation, an incarcerated household member, and household substance use), before the age of 18 (Dong et al., 2004). Additionally, as the number of domains of ACEs increased, the number of health risk behaviors (e.g., excessive alcohol use; Dube et al., 2002) as well as mental and physical health outcomes also increased, in a graded "dose-response" fashion (Anda et al., 2006).

Adverse Childhood Experiences

Early life stress is thought to take a cumulative toll on an individual's health, whereby there is a graded relationship between ACE exposure and prevalence and

severity of negative health outcomes (Anda et al., 2006; Felitti et al., 1998; Campbell et al., 2016; Danese et al., 2009; Voellmin et al., 2015). Therefore, each additional domain of ACE increases the risk and severity of health outcomes in a graded fashion, beyond the sum of each domain of ACE alone. Additionally, domains of ACEs tend to co-occur (Edwards et al., 2003). That is, an individual who has experienced one domain of ACE is more likely to have experienced multiple domains of ACEs, possibly having grown up in a chronically chaotic and unsupportive household. Therefore, such individuals may be at risk for increased and more severe future health outcomes. For example, research indicates that adult children of alcoholics (i.e., those who grew up with one or both parents experiencing alcoholism) have the highest likelihood of additional ACEs as compared to those without a parental history of alcoholism (Dube et al., 2001). Thus, adult children of alcoholics may be at an increased risk for the cumulative impact of early life stressors.

Although changing due to public awareness of ACEs, early life stress was not traditionally recognized to be associated with subsequent outcomes (Felitti, 1993; Felitti et al., 1998). Even after two decades of ACE research, many healthcare professionals may still lack familiarity with ACEs (Stork et al., 2020). Therefore, this relationship may not be realized until health risk behaviors and overt mental and physical health consequences emerge. For example, results from the original ACE Study, and others, indicate that the sum of the domains of ACEs had a strong, graded relationship to the prevalence of a constellation of health outcomes. Specifically, the relationship between ACEs and substance use outcomes has been established for smoking (Anda et al., 1999; Campbell et al., 2016; Elliott et al., 2014; Ford et al., 2011), alcohol use, (Anda et al.,

2002; Campbell et al., 2016; Chatterjee et al., 2018; Dube et al., 2002; Dube et al., 2006; Elliott et al., 2014; Pilowsky et al., 2009), marijuana use (Chatterjee et al., 2018), prescription drug use (Anda et al., 2008), illicit drug use (Dube et al., 2003), and intravenous drug use (Anda et al., 2006). It is suggested that changes to the stress response system and reward pathways subsequent to early life stress may constitute a vulnerability to future substance use (Moustafa et al., 2021). While research seems to support a pathway from early life stress to future substance use by means of physiological changes, protective factors accounting for resilient outcomes have also been noted (Enoch, 2011).

Hypothalamic-Pituitary-Adrenal Axis

It is hypothesized that aberrant functioning of the hypothalamic-pituitary-adrenal (HPA) axis is one of the pathways through which early life stress leads to negative physical health outcomes, mental health challenges, and risk-taking behaviors, including substance use. Prolonged dysregulation of the HPA axis may cause structural and functional alterations in the limbic system and other relevant pathways. Such alterations may constitute a vulnerability to negative mental health and behavioral outcomes including emotion dysregulation, affective disorders, and health risk behaviors. While the inclusion of negative physical health outcomes, mental health challenges, and risk-taking behaviors is warranted in research involving ACEs and biomarkers of stress, these additional variables are outside of the scope of the proposed study. Rather, the focus of this investigation was narrowed to the assessment of ACEs, symptoms and consequences of substance use, and cortisol awakening response.

The HPA axis affects the neuroendocrine system through multiple mechanisms to promote allostasis (i.e., “stability through change”; Sterling & Eyer, 1988) when faced with stress, thereby contributing to the impact of ACEs on the developing brain (Danese & McEwen, 2012). The process of stress system response begins with threat appraisal and processing in the amygdala, orbital and medial prefrontal cortex (PFC), cingulate cortex, and locus coeruleus (Tarullo et al., 2008). The central amygdala and lateral bed nucleus of the stria terminalis (BNST) signal the medial parvocellular region of the paraventricular nucleus (mpPVN) of the hypothalamus to secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP; Tarullo and Gunnar, 2006). Then, CRH binds in the anterior pituitary and stimulates the release of adrenocorticotropic hormone (ACTH). Next, ACTH travels through the blood and binds in the adrenal cortex, which stimulates the release of stress hormones, including glucocorticoids (Lupien et al., 2007; Tsigos & Chrousos, 2002). For the purposes of this study, cortisol is the most relevant of these hormones.

Glucocorticoids also play an inhibitory role in the HPA axis through negative feedback loops, whereby the secretion of CRH and ACTH becomes downregulated (Charmandari et al., 2005; Tsigos & Chrousos, 2002). Consequently, it is plausible that cortisol levels become blunted over time following chronic HPA axis activation (Danese & McEwen, 2012). In support of this notion, researchers found a significant positive correlation between overall cortisol level (i.e., mean across four times of day for three consecutive days) and posttraumatic stress disorder (PTSD) symptoms in children who had experienced a traumatic event within the last year (Weems & Carrion, 2007). Yet, these authors reported a non-significant negative correlation between overall cortisol

level and PTSD symptoms in children whose traumatic experience had occurred over a year prior.

Additionally, results of a longitudinal study indicated that the morning cortisol levels in females who had experienced sexual abuse were initially significantly higher than those of the healthy controls (Trickett et al., 2010). Further, these researchers found that cortisol levels began to decrease in adolescence and were significantly lower in early adulthood in abused females, as compared to controls. Notably, serum assessment of cortisol was used for the first three time points of the study, and salivary assessment of cortisol was used for the following three time points of the study, and these measurements are shown to be strongly correlated (Bober et al., 1988). The results of Trickett and colleagues (2010) indicate that, in a normal course of development, cortisol may steadily increase from middle childhood into early adulthood, and then plateau. The results support the notion that children who endure ACEs may experience an initial hypersecretion of cortisol followed by a hyposecretion of cortisol. Other researchers have found that, in young adult women free of illegal drug use or heavy tobacco use (i.e., five or more cigarettes per day), the duration of ACE exposure was associated with attenuated cortisol responses to a stress task in a dose-response fashion, consistent with the aforementioned concept (Voellmin et al., 2015). In addition, Kuras and colleagues (2017) found that, in a sample with no psychoactive drug use or smoking, participants who reported low to moderate childhood adversity exhibited blunted morning cortisol and diurnal slope compared to those who reported no adversity. However, no such difference was observed in CAR, which the authors calculated using area under the curve with respect to ground (AUC_G). Taken together, the research suggests that adverse childhood

experiences may have a long-term effect on HPA axis functioning that may persist into adulthood.

Measurement of Cortisol

Cortisol is a commonly utilized and minimally invasive biomarker of HPA axis functioning. One method of assessing cortisol response is by collecting samples before, during, and following an acute psychosocial stressor paradigm, and this method has been historically conducted in a laboratory setting (e.g., Kirschbaum et al., 1993). An alternative to the aforementioned measure is the ambulatory collection of samples in assessing cortisol awakening response (CAR) and/or diurnal slope, which represent chronic, daily stress (for review, see Saxbe, 2008). Cortisol levels exhibit a diurnal rhythm, whereby in healthy individuals, basal cortisol levels peak about 30 minutes after waking and gradually decline across the day (Fries et al., 2009). Dysregulated CAR has been implicated in clinical and subclinical disorders, physical health problems, as well as chronic stress and burnout, among others (Saxbe, 2008).

Factors That Influence Cortisol Response

Several factors have been shown to influence cortisol levels and should be accounted for when assessing this biomarker. For example, sex differences in cortisol reactivity to acute stressors are well documented, with men exhibiting a higher response compared to women (Kirschbaum & Hellhammer, 1992; Kudielka & Kirschbaum, 2005; Liu et al., 2017). Perhaps this differential HPA axis response reflects greater vulnerability to stress-related psychological disorders in women than in men (Kudielka & Kirschbaum, 2005). However, other researchers found that women exhibited a greater CAR than men did by 20% (Almeida et al., 2009). In addition, research indicates that women who use

oral contraceptives (OC) may show an increase in free circulating cortisol levels (Hertel et al., 2017; Pruessner et al., 1997). However, authors reported a very small effect size, with OC use accounting for only 4% of variance in CAR (Pruessner et al., 1997).

Further, the relationship between CAR and affective disorders is mixed. For example, Bhagwagar and colleagues (2005) found that those experiencing clinical depression exhibited a hypersecretion in CAR as compared to healthy controls. In addition, the association between clinical depression and heightened cortisol response following an acute momentary stressor has been found consistently across studies (Burke et al., 2005). However, researchers have found both increased and blunted CAR to be associated with depression across studies, possibly due to inconsistent measurements of depression severity and symptomatology (Dedovic & Ngiam, 2015). Additionally, Walker and colleagues (2011) found that those high in trait anxiety had a decreased CAR compared to those low in trait anxiety. Similarly, research indicates that high loneliness scores are associated with blunted CAR (Lai et al., 2018). These findings suggest that participants' sex assigned at birth, use of oral contraceptives, as well as emotional functioning (i.e., depression, anxiety, and loneliness symptoms) should be included as covariates in studies including the measurement of diurnal cortisol.

Interestingly, researchers found that high CAR levels at age 17 were predictive of new or recurrent major depressive episodes within 2.5 years following the initial measurement (Vrshek-Shallhorn et al., 2013). In addition, researchers of the same project found that high CAR levels at age 17 were also predictive of first onset of social anxiety disorder up to four years from the initial cortisol measure (Adam et al., 2014). These

findings suggest the possibility that high CAR levels may represent a direct or indirect risk factor for depressive and anxiety disorders, rather than a consequence thereof.

Prevalence Rates

Substance Use among College-Age and Young Adults

Results from the National Epidemiologic Survey on Alcohol and Related Conditions III suggest that among young adults ages 18 to 29, the 12-month rate of heavy drinking was 68% for men and 48% for women (Grant et al., 2015). The researchers additionally found that there was a lifetime prevalence of alcohol use disorder of 37% in this same age group.

Among college students at a large, mid-Atlantic university, researchers found a lifetime prevalence of 68% for marijuana use, 34% for prescription stimulant use for non-medical purposes, 28% for prescription analgesic use, 21% for hallucinogen use, 17% for prescription tranquilizer use, and 17% for cocaine use (Arria et al., 2017). In this study, lifetime use was measured by asking participants to report how many times they had used each category of drug during their lifetime. Other research indicates more conservative prevalence rates among college students. Results of the national Monitoring the Future survey showed that, among college students in 2019, there was a lifetime prevalence of 54% for marijuana use, 14% for prescription amphetamine use not under a doctor's orders, 6% for narcotic use other than heroin, 9% for hallucinogen use, 7% for tranquilizer use, and 9% for cocaine use (Schulenberg et al., 2020). As a comparison, results of the same national survey indicated that, among young adults ages 19 to 30, there was a lifetime prevalence of 64% for marijuana use, 20% for prescription

amphetamine use not under a doctor's orders, 13% for narcotic use other than heroin, 15% for hallucinogen use, 12% for tranquilizer use, and 15% for cocaine use.

Additionally, 8% of college students and 12% of young adults ages 19 to 28 reported cigarette use in the past 30 days, and another 16% of college students and 22% of young adults ages 19 to 28 reported past-year cigarette use (Schulenberg et al., 2020). An additional 7% reported using smokeless tobacco in the past 30 days and 23% reported lifetime use of smokeless tobacco in the same survey.

In addition, e-cigarette initiation (Sutfin et al., 2015) and use (Littlefield et al., 2015) are becoming increasingly prevalent among college students. Research has shown that 14% of college students reported past 30-day e-cigarette use, and 29% report lifetime e-cigarette use (Littlefield et al., 2015). More recent research found that 29% of college students reported past 30-day vaping and 49% reported lifetime vaping in 2019 (Schulenberg et al., 2020). Additionally, e-cigarette use is associated with heavy alcohol use (Littlefield et al., 2015) and is shown to be a significant risk factor for future cigarette use in college students (Loukas et al., 2018).

Adverse Childhood Experiences among College Students

There is variability in the reported prevalence rates of ACEs, early life stress, and childhood maltreatment across various studies of populations of interest. It is evident that additional research is necessary for an accurate representation of prevalence rates of ACEs and associated outcomes. For example, one investigation found that 53% of the sample of college students in the state of Minnesota endorsed having had at least one ACE (Karatekin, 2017). In a large sample of racially/ethnically diverse college students in Minnesota, researchers found that 45% of students reported having a history of at least

one ACE (Forster et al., 2019), compared to 57% in a subsequent study by the same researchers (Grigsby et al., 2020). The divergent results of the aforementioned studies can be compared to the 64% of middle-aged respondents in the original ACE Study who reported having a history of at least one ACE (Anda et al., 2006). It should be noted that researchers have found good to excellent consistency in evaluating retrospectively reported ACEs among adolescents (M_{age} = 17) with a documented history of childhood maltreatment (Pinto et al., 2014). Thus, perhaps the lower frequency of reported ACEs in college students compared to that of the middle aged adults in the original ACE Study is a reflection of a more privileged background in college students (Karatekin, 2017).

Among college students who have endured ACEs, the co-occurring mental health outcomes and continued familial disruption may represent a barrier to academically thriving (Hinojosa et al., 2019). Specifically, Hinojosa and colleagues (2019) found that college students with higher levels of ACE exposure more frequently report impediments to academic success, including alcohol use, other substance use, caregiving for a parent, partner, or child, difficulty in family relationships, health problems, as well as symptoms of depression. In addition, the researchers reported that students with higher exposure to ACEs also indicated more difficulty managing time, existing learning difficulties, as well as incongruent teaching and learning styles. Additionally, other researchers have found that college students who reported higher levels of ACEs were more likely to report mental health outcomes and health-compromising behaviors, including higher levels of depressive and ADHD symptoms, less hours of sleep, higher body mass index (BMI), less fruit and vegetable consumption, as well as higher levels alcohol, marijuana, and cigarette use (Windle et al., 2018). Therefore, additional research pertaining to ACEs and

substance use in a college population is merited, as it could inform approaches to promoting academic success.

Adverse Childhood Experiences and Substance Use

Several studies have assessed the relationship between ACEs and substance use among college students. Forster and colleagues (2019) found that college students of color reported higher levels of ACEs than did white students, but that white students reported higher substance use behaviors than students of color did.

In addition, researchers have found that ACEs were a risk factor for substance use behaviors including cigarettes (Forster et al., 2019; Grigsby et al., 2020), e-cigarettes (Grigsby et al., 2020), alcohol (Forster et al., 2019), binge drinking (Forster et al., 2019; Grigsby et al., 2020), marijuana (Forster et al., 2019), and illicit substance use (Forster et al., 2019) in college students. Additionally, like the original ACE Study, researchers found a dose-response relationship between exposure to ACEs and endorsement of substance use behaviors in college student samples (Forster et al., 2019; Grigsby et al., 2020).

Adverse Childhood Experiences, Cortisol, and Substance Use

Few studies have examined the relationship between ACEs and cortisol using a psychosocial stressor paradigm, and these studies reported divergent cortisol responses (i.e., hypo- or hyper-secretion) based on sex and substance use status. For example, Hood and colleagues (2020) found that, following a psychosocial stressor paradigm, higher ACE scores were associated with heightened cortisol response in male smokers and attenuated cortisol response in female smokers. In a separate study of healthy female non-substance users, researchers found that higher ACE scores were associated with

attenuated cortisol response following a psychosocial stressor task (Voellmin et al., 2015). Although the research is sparse, it appears that higher ACE scores are associated with lower acute cortisol response in females and higher acute cortisol response in males, regardless of substance use status (Hood et al., 2020; Kuras et al., 2017; Trickett et al., 2010; Voellmin et al., 2015). However, there is a gap in the literature pertaining to CAR in the context of ACEs and risky substance use in college-aged adults. The current study aimed to address this limitation and may inform practices for identifying students at risk for hazardous substance use by means of self-collected cortisol samples.

The Current Studies

The purpose of the current studies was to assess substance use behaviors, retrospective adverse childhood experiences, and cortisol awakening response. The specific aims of this project included determining the relationship between exposure to ACEs and risky substance use, determining whether perceived stress mediates the relationship between exposure to ACEs and risky substance use, determining whether the diurnal cortisol rhythm varies by substance use status and sex, and determining whether CAR varies by ACE exposure.

Study One Hypotheses:

H1. Perceived stress levels were expected to be higher among hazardous substance users than non-hazardous substance users, and higher among females compared to males.

H2. It was expected that there would be a significant positive relationship between exposure to ACEs and symptoms and consequences of substance use.

H3. It was predicted that that perceived stress levels would mediate the relationship between cumulative exposure to ACEs (i.e., ACE score) and consequences of alcohol use.

Study Two Hypotheses:

H4. It was expected that the diurnal cortisol levels would be lower among females and higher among males regardless of substance use status.

H5. It was expected that ACE score would significantly predict CAR.

Study One

Method

Participants

A total of 271 college students were recruited for Study One. A total of six participants were removed from the data set for the following reasons: five responded incorrectly to one of three attention check items, and one gave spurious responses. Thus, the final sample was comprised of 265 adults with a mean age of 19.24 years (*SD* = 1.52; range = 18-30 years). One hundred and forty-three participants identified as women (54.0%), 115 as men (43.4%), five as gender minorities (1.9%), and two did not disclose (0.8%). Of these, 54.4% were assigned female at birth, 45.2% were assigned male at birth, and 0.4% did not disclose. The majority of participants identified as white (86.8%) and heterosexual (82.4%) and held freshmen status (60.8%).

Primary Measures

Demographic Questionnaire. A demographic questionnaire was administered and included standard items such as age, gender identity, use of oral contraceptives,

race/ethnicity, sex, sexual orientation, year in school, height and weight to calculate BMI, and other pertinent items.

Adverse Childhood Experiences. The ACE Study questionnaire (ACE-Q; Dong et al., 2004) is a 10-item questionnaire that was administered to assess adverse life events that occurred before the participant was 18 years of age. This questionnaire assessed retrospective experiences of childhood abuse (emotional, physical, sexual), neglect (emotional and physical), and household dysfunction (mother treated violently, household substance use, household mental illness, parental separation or divorce, incarcerated household member). For each ACE variable, endorsement of a *yes* response for having been exposed to a category of childhood adversity was coded as 1, and endorsement of a *no* response was coded as 0 (Dong et al., 2004). Then, a sum was computed to determine the ACE score; possible scores range from 0-10. The ACE-Q demonstrated acceptable internal consistency ($\alpha = .77$).

Symptoms and Consequences of Substance Use. The Young Adult Alcohol Consequences Questionnaire (YAACQ; Read et al., 2006) is a 67-item questionnaire that was utilized to assess eight domains of past-year consequences of drinking. These domains include social/interpersonal consequences, academic/occupational consequences, risky behavior, impaired control, poor self-care, diminished self-perception, blackout drinking, and physiological dependence. Items were measured dichotomously, with responses of *yes* being coded as 1, and responses of *no* being coded as 0. Sample item includes, “I often drank more than I originally had planned.” Established cutoff scores were used to group those at low (0-7 for men; 0-9 for women), moderate, (8-16 for men; 10-16 for women), and high (≥16 for both genders) risk of

hazardous drinking (Read et al., 2016). Given that the YAACQ demonstrated excellent internal consistency (α = .96), this measure was used as the primary index of hazardous alcohol use in analyses.

The Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001) is a 10-item questionnaire that was used to evaluate symptoms of hazardous drinking and active disordered alcohol use, including alcohol abuse and dependence. Items were scored on a scale of 0 to 4 with higher scores indicative of a pattern of use that may affect an individual's health or safety. The AUDIT demonstrated good internal consistency (α = .84).

Daily Drinking Questionnaire (DDQ; Collins et al., 1985;) was used to determine binge drinking status (i.e., five or more drinks on one occasion for males and four or more drinks on one occasion for females; National Institute on Alcohol Abuse and Alcoholism [NIAAA], 2004) and heavy drinking status (i.e., 15 or more drinks per week for males and 8 or more drinks per week for females; NIAAA). The DDQ demonstrated acceptable internal consistency (α = .77).

The Marijuana Problem Inventory (MPI; Vandrey et al., 2005) is a 29-item survey that was used to assess problem consumption of marijuana. Items were adjusted to reflect any consumption of THC products (e.g., edibles, vapes, tinctures). Items were scored as frequencies within the past year (i.e., *0 times* through *more than 10 times*). Higher scores are indicative of higher problematic outcomes. Sample item includes, "Went to school high or stoned." The MPI demonstrated excellent internal consistency (α = .94).

The Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991) and its adapted versions for e-cigarettes (e-FTND; Etter & Eissenberg, 2015) and

smokeless tobacco (FTND-ST; Ebbert et al., 2006) were administered to assess physical dependence on nicotine. Each 6-item inventory was used to assess physical dependence for respective tobacco products. Four dichotomous items were scored from 0 to 1, and two multiple choice items were scored from 0 to 3. The sum of scores for each inventory ranges from 0-10. For each category of nicotine product, a score above 5 is indicative of significant dependence, and a lower score is indicative of low to moderate dependence. The e-FTND demonstrated questionable internal consistency ($\alpha = .61$).

The Perceived Stress Scale (PSS; Cohen et al., 2018) is a 14-item inventory that was used to assess the extent to which individuals perceive past-month life circumstances as uncontrollable, unpredictable, or overloading. Further, perceived stress as measured by the PSS is shown to be an accurate predictor of diurnal cortisol slope (Knight et al., 2021). The PSS demonstrated good internal consistency ($\alpha = .80$).

Secondary Measures

The Spielberger State Anxiety Inventory (STAI; Spielberger et al., 1983) is a 20-item questionnaire that was administered to measure state anxiety. This assessment includes items such as, “I feel tense,” “I am worried,” and “I feel calm.” Items were scored on a scale of 1 to 4, with a score of 4 indicating that the participant *very much agree[s]* with the respective statement. The STAI demonstrated excellent internal consistency ($\alpha = .93$).

The Center for Epidemiologic Studies Depression (CES-D; Radloff, 1977) is a 20-item questionnaire that was used to assess self-reported levels of depression within the past week. Items were assessed on a scale of 0 to 3, with 0 indicating *rarely* and 3 indicating *most or almost all the time*. The possible range is 0 to 60. Sample item

includes, “I thought my life had been a failure.” The CES-D demonstrated excellent internal consistency (α = .91).

The revised UCLA Loneliness Scale (Russell et al., 1980) is a 20-item inventory that was administered to assess self-reported loneliness and social isolation. Items were scored on a scale of 0 to 3, with 0 indicating *I never feel this way* to 3 indicating *I often feel this way*. Sample item includes, “It is difficult for me to make friends.” The revised UCLA Loneliness Scale demonstrated excellent internal consistency (α = .93).

The Risky, Impulsive, and Self-Destructive Behavior Questionnaire (RISQ; Sadeh & Baskin-Sommers, 2016) is a 38-item questionnaire that was used to assess the severity, chronicity, and affective triggers for eight domains of risky behavior. These domains include drug use, aggression, self-harm, gambling, risky sexual behavior, impulsive eating, heavy alcohol use, and reckless behavior. Number of times of engagement in each behavior was measured as a continuous variable. Interference with personal life was measured dichotomously, with responses of *yes* being coded as 1, and responses of *no* being coded as 0. Affective motivations (i.e., approach and avoidance) for each behavior was scored on a scale of 0 to 4 with higher scores indicative of strong endorsement of the respective motivation.

Finally, the Life Events Questionnaire (LEQ; Norbeck, 1984; Sarason et al., 1978) was used to assess individuals’ experiences of a number of past-year life events, the effect (positive or negative) of each event, as well as the extent of impact (i.e., *no effect* to *great effect*); several items excluded for the purposes of this study. Analyses were not conducted using the secondary measures as these indices were beyond the scope of the research questions of Study One.

Procedure

Participants were recruited from a large Midwestern university using the online research recruitment tool, SONA®. Prospective participants were given access to a survey link hosted on Qualtrics®, and participants were given additional information about the study's purpose prior to providing informed consent. Consenting participants were then asked to complete the series of questionnaires, which took approximately 30 to 40 minutes. Participants were granted course credit for their participation. This study was approved by the University's institutional review board prior to data collection.

Results

Data were screened and cleaned in accordance with procedures outlined by Tabachnick and Fidell (2013). Pairwise deletion was used to deal with missing values in order to ensure the most robust sample size when conducting analyses.

Descriptive Statistics

See Table 1 for prevalence of each category of adverse childhood experience and cumulative exposure to ACEs (i.e., ACE score). Overall, 88.8% of respondents reported ever consuming alcohol (i.e., more than a few sips), with a mean age of first use of 16.59 (*SD* = 1.57) years. Of those who have consumed alcohol, 59.7% met criteria for hazardous drinking using cutoff scores for symptoms of active disordered alcohol use established by the AUDIT (Babor et al., 2001), while 51.1% of the full sample (i.e., including those who have not consumed alcohol) met criteria for hazardous drinking. In addition, 42.1% of respondents reported moderate to high risk for hazardous drinking in the past year using established cutoff scores in accordance with the YAACQ (Read et al., 2006; 2016). Additionally, 33.5% of respondents reported current use of marijuana and/or

THC products with a mean age of first use of 16.61 (*SD* = 1.72) years. Further, 24.3% of respondents reported current use of e-cigarettes with a mean age of first use of 15.82 (*SD* = 1.35) years. Given that only 2.8% and 6.8% of respondents reported current cigarette and smokeless tobacco use, respectively, analyses were not conducted using these variables.

Primary Analyses

Perceived Stress by Substance Use Status and Sex. To assess the hypothesis that perceived stress levels would differ by substance use status, as well as by sex assigned at birth, several analyses of variance (ANOVAs) were conducted to assess main effects and interactions on perceived stress levels. For this analysis, the YAACQ and its established cutoff points were used, as this measure displayed the highest reliability of the scales that assessed symptoms and consequences of alcohol use. First, a 3 (high, moderate, low risk of hazardous drinking; YAACQ) × 2 (male, female) ANOVA was conducted with perceived stress (PSS) as the dependent variable. The results revealed that, although the interaction was not significant ($F[2, 241] = 1.91$, $p = .15$, $\eta p^2 = 0.02$), the univariate effects of alcohol risk status, $F(2, 241) = 3.78$, $p = .02$, $\eta p^2 = 0.03$, and sex, $F(1, 241) = 4.89$, $p = .03$, $\eta p^2 = 0.02$, were significant. A follow-up examination of the descriptive statistics revealed that males ($M = 26.46$, $SE = 0.72$) reported lower stress levels in comparison to females ($M = 28.65$, $SE = 0.68$) and that those at high risk for hazardous drinking reported greater stress levels ($M = 29.43$, $SE = 0.99$) in comparison to those at low risk for hazardous drinking ($M = 26.27$, $SE = 0.58$).

Next, a 2 (high, low e-cigarette dependence; e-FTND) × 2 (male, female) ANOVA was conducted among those who currently use e-cigarettes, with perceived

stress (PSS) as the dependent variable. The results indicated that, while the interaction (F[1, 55] = 0.12, p = .72, ηp^2 = 0.002) was not significant and the univariate effect of e-cigarette status (F[1, 55] = 3.99, p = .05, ηp^2 = 0.07) was marginally significant with a medium effect size, the univariate effect of sex was significant, F(1, 55) = 4.53, p = .04, ηp^2 = 0.08. A follow-up examination of the descriptive statistics revealed that those with low e-cigarette dependence (M = 27.14, SE = 1.23) reported lower stress levels than those with higher dependence (M = 30.51, SE = 1.15) and that males (M = 27.03, SE = 1.28) reported lower stress levels in comparison to females (M = 30.62, SE = 1.09).

Lastly, a 4 (never, past, current non-daily, current daily THC/marijuana use) × 2 (male, female) ANOVA was conducted with perceived stress (PSS) as the dependent variable. The results revealed that, although the interaction was not significant (F[3, 241] = 0.29, p = .83, ηp^2 = 0.004), the univariate effects of THC use status, F(3, 241) = 2.32, p = .03, ηp^2 = 0.03, and sex, F(1, 241) = 5.62, p = .02, ηp^2 = 0.02, were significant. A follow-up examination of the descriptive statistics revealed that males (M = 26.42, SE = 0.71) reported lower stress levels in comparison to females (M = 28.76, SE = 0.68) and that daily THC users reported greater stress levels (M = 29.30, SE = 1.30) in comparison to those who reported never using THC products (M = 25.81, SE = 0.73).

Relationship Between ACEs and Substance Use. To assess the hypothesis that there would be a significant positive relationship between exposure to ACEs and symptoms and consequences of substance use, Pearson's correlation coefficients were computed between ACE score and substance use scales. Results revealed positive correlations between ACE score and consequences of alcohol use (YAACQ composite score; r[246] = .18, p < .01) and between ACE score and e-cigarette dependence (eFTND

composite score; $r[57] = .31$, $p = .02$) but not between ACE score and consequences of THC use (MPI composite score; $r[153] = .11$, $p = .19$).

Role of ACEs and Perceived Stress in Substance Use. Because of the difference in perceived stress levels between those at high and low risk for alcohol-related consequences and because of the significant positive relationship between ACE score and alcohol-related consequences, a mediational analysis was conducted in order to assess the hypothesis that perceived stress levels would mediate the relationship between cumulative exposure to ACEs (i.e., the ACE score) and substance use outcomes (i.e., YAACQ cumulative score). The mediational model was tested using Hayes' PROCESS Macro (Model 4; Hayes, 2013). The requirements for concluding significant mediation were: (1) the mediator (M) is significantly associated with the predictor variable (X) and the outcome variable (Y), (2) the direct effect is significantly different from zero, and (3) the indirect effect of X →Y via M is statistically different from zero (Preacher & Hayes, 2008). To assess the significance of the indirect effect (c-c′), confidence intervals were reported.

In this model, ACE score was included as the predictor variable (X), consequences of drinking (i.e., YAACQ composite score) as the outcome variable (Y), and perceived stress (i.e., PSS composite score) as the mediator (M). The results of the mediational analysis indicated that perceived stress mediated the effect of ACE score on the extent to which individuals reported consequences of drinking (See Figure 1). After perceived stress was added to the model, the coefficient was reduced from $\beta = 0.92$ to $\beta = 0.79$. The indirect effect of ACE score on consequences of drinking through perceived

stress was significantly different from zero, indirect effect = 0.13, *SE* = 0.07, 95% CI = [0.01, 0.28].

Study Two

Method

Participants

A total of 55 participants were recruited to participate in Study Two. Mean age of the participants was 19.53 years (*SD* = 1.65; range = 18-26 years). Twenty-nine participants were assigned male at birth (52.7%), and 26 were assigned female at birth (47.3%). The majority of participants identified as white (81.8%) and heterosexual (73.6%) and held freshmen status (48.1%).

Primary Measures

Demographic Questionnaire. A demographic questionnaire was administered and included standard items such as age, gender identity, use of oral contraceptives, race/ethnicity, sex, sexual orientation, year in school, height and weight to calculate BMI, and other pertinent items. Oral contraceptive use and BMI were originally intended to be used as covariates but were excluded from analyses given lack of sufficient power.

Adverse Childhood Experiences. The ACE-Q (Dong et al., 2004) described in Study One was used to assess adverse life events that occurred before the participant was 18 years of age. ACE score was computed as a continuous variable with possible scores ranging from 0-10. To examine group differences, participants were grouped by those who reported low (0 to 1) and high (≥2) ACE categories (Karatekin, 2017). The ACE-Q demonstrated questionable internal consistency (α = .66).

Symptoms and Consequences of Substance Use. For the purposes of this study, hazardous substance users were defined as those who meet criteria for hazardous drinking as defined by the YAACQ (Read et al., 2006) and by the AUDIT (Babor et al., 2001) and who engage in heavy episodic drinking as measured by the DDQ (Collins et al., 1985), as described in Study One. Internal consistency for the YAACQ was excellent ($\alpha = .95$) and for the AUDIT was acceptable ($\alpha = .79$); thus, the YAACQ was used for primary analyses in Study 2. Specifically, established cutoff scores for the YAACQ were used to group those at low (0-7 for men; 0-9 for women) and moderate to high ($\geq$8 for men; $\geq$10 or women) risk of hazardous drinking (Read et al., 2016). The MPI (Vandrey et al., 2005) described in Study One was used to assess marijuana-related consequences. The MPI demonstrated excellent internal consistency ($\alpha = .91$). The FTND, as described in Study One, was used assess symptoms of physical dependence to cigarettes (Heatherton et al., 1991), e-cigarettes (Etter & Eissenberg, 2015), and smokeless tobacco (Ebbert et al., 2006).

Cortisol Awakening Response. The self-administered ambulatory assessment of CAR is well established and is used as an indicator of stress system (i.e., HPA axis) activity (Saxbe, 2008). The current study implemented protocol specified by Salimetrics, LLC, specifically using Salivary Oral Swabs as the collection instrument. Participants collected four samples at home across 1 day (i.e., immediately after awakening, 30 minutes post-awakening, 60 minutes post-awakening, and at approximately 8:00 p.m.). Previous researchers have found high protocol compliance of in-home collection of cortisol and suggest the feasibility of naturalistic CAR collection procedures (Walls et al., 2020). Participants refrigerated their samples in their place of residence until retrieved by

the investigator after the sample collection day, as salivary cortisol samples are shown to be stable for at least 72 hours at room temperature (Nalla et al., 2015). Finally, samples were centrifuged and stored in a -80°C freezer onsite until shipped to Salimetrics, LLC, where cortisol levels were quantified.

Following collection of each sample, participants completed a brief subjective state survey via Qualtrics which was accessed by following a link sent in a text message. This survey was based on factors outlined by Linz and colleagues (2018) and controlled for current emotional distress, physical symptoms of stress, food and beverage consumption, physical exercise, company of others, and true collection time. The subjective state survey included the Positive and Negative Affect Scale (PANAS; Watson et al., 1988; $\alpha = .77$ to .83 across four time points). This instrument is a 20-item inventory in which items were scored on a scale of 1 to 5. Higher numbers indicate a greater extent to which an individual reported having felt each emotional word (e.g., “distressed,” “excited”).

Secondary Measures

The STAI (Spielberger et al., 1983) described in Study One was used to measure state anxiety; internal consistency was excellent ($\alpha = .93$). The CES-D (Radloff, 1977), as described in Study One, was used to assess self-reported depression symptoms within the past week; internal consistency was good ($\alpha = .89$). The revised UCLA Loneliness Scale (Russell et al., 1980) was administered to assess self-reported loneliness and social isolation, as described in Study One; internal consistency was excellent ($\alpha = .93$). These three measures (i.e., the STAI, CES-D, and revised UCLA Loneliness Scale) were not included as covariates given a lack of sufficient power for the main analyses.

In addition, the RISQ (Sadeh & Baskin-Sommers, 2017) was used to assess the severity, chronicity, and affective triggers for eight domains of risky behaviors. Analyses were not conducted using the RISQ as this measure was beyond the scope of the research questions of Study Two.

Procedure

Participants were recruited from a large Midwestern university using flyers around campus and the online research recruitment tool, SONA®. Prior to beginning the study, participants were screened for eligibility. Exclusionary criteria included current use of medication containing a corticosteroid, diagnosed neuroendocrine disorder (e.g., Addison's or Cushing's diseases), recent illness, or if the individual was not proficient in English. In addition, consenting participants were provided with comprehensive verbal and written instructions about the importance of adhering to sampling procedures (e.g., scheduling bedtime between 9:00 p.m. and 12:00 a.m. the night before collection; scheduling wake time between 6:00 a.m. and 8:00 a.m. the day of collection; refraining from brushing teeth, eating, consuming caffeine, or smoking a cigarette prior to sample collection). Participants were compensated with their choice of a $15.00 prepaid gift card or course credit for their participation. This study was approved by the University's institutional review board prior to data collection.

Trained research assistants described the study and provided consent forms to potential participants on-site. Consenting participants were screened for eligibility using a structured interview. Those who met eligibility requirements were then provided with comprehensive written and verbal instructions about the sampling protocol. Participants were told the ways in which they could be compensated for their participation. In

addition, participants were each provided with four saliva collection tubes and Salivary Oral Swabs to account for four collection times across one day. These tubes were labeled with participant number as well as the respective collection dates and times.

Participants were sent text message reminders prior to each sample collection time, completed salivary cortisol samples in accordance with sampling procedures, and, following each sample, completed the subjective state survey from their phones. Participants refrigerated their samples in their residence until returned to research assistants after the collection day. Samples were then centrifuged and stored in a -80°C onsite freezer at the University of Minnesota Duluth, where samples remained until shipped to Salimetrics, LLC for quantification. After participants returned their samples, they were asked to complete a battery of questionnaires and provided with a link to a survey hosted on Qualtrics®. The survey took approximately 15 to 30 minutes to complete.

Results

Data were screened and cleaned in accordance with procedures outlined by Tabachnick and Fidell (2013). The average of five imputed values was used in place of one female participant's fourth cortisol sample, as the original quantification was an outlier (i.e., 7 standard deviations above the mean) for that data point. One female participant's cortisol levels were extreme outliers (i.e., 4 to 7 standard deviations above the mean) at all four time points and were thus excluded from analyses. Possible reasons for the observed outliers may include tempering or contamination during the collection or analysis process, an undiagnosed or undisclosed health concern, or undisclosed medication use.

Additionally, one male participant completed all cortisol samples but failed to complete the survey portion of the study. An additional seven participants did not adhere to study protocol. Specifically, these "non-adherers" completed their first saliva sample between 25 and 120 minutes after awakening rather than immediately after awakening (i.e., six males and one female); thus, their cortisol data were excluded from analyses. However, even after excluding those whose first sample was delayed, nine participants (16.7%) who adhered to study protocol still failed to display an increase in cortisol from Time 1 to Time 2, resulting in a negative T2 to T1 difference; these "CAR non-responders" were retained in the sample to reduce bias, as there is some evidence to suggest that on a minority of days (i.e., 14.7% to 19.7%), adults may present as CAR non-responders (Dockray et al., 2008; Smyth et al., 2013). Pairwise deletion was used to deal with missing values in order to ensure the most robust sample size when conducting analyses.

Descriptive Statistics

Of the 55 participants, the majority (80%) were recruited from SONA or class announcements and received course credit for their participation, while the remainder received gift cards as compensation for their participation. See Table 2 for prevalence of each category of adverse childhood experience and cumulative exposure to ACEs (i.e., ACE score). Overall, 85.2% of respondents reported ever consuming alcohol (i.e., more than a few sips), with a mean age of first use of 16.67 (*SD* = 1.65) years. Of those who have consumed alcohol, 29.8% met criteria for hazardous drinking using cutoff scores for symptoms of active disordered alcohol use established by the AUDIT (Babor et al., 2001), while 25.5% of the full sample (i.e., including those who have not consumed

alcohol) met criteria for hazardous drinking (M = 4.29; SD = 4.47). In addition, 37.0% of respondents reported moderate to high risk for hazardous drinking in the past year using established cutoff scores in accordance with the YAACQ (Read et al., 2006; 2016). Further, 46.8% of participants met criteria for binge drinking using the DDQ. Additionally, 25.9% of respondents reported current use of marijuana and/or THC products with a mean age of first use of 16.72 (SD = 1.69) years. Further, 10.9% of respondents reported current use of e-cigarettes with a mean age of first use of 15.96 (SD = 3.93) years. Given that only 3.7% and 1.85% of respondents reported current cigarette and smokeless tobacco use, respectively, analyses were not conducted using these variables.

After excluding those who did not adhere to study protocol, the mean amount of time between waking and completing the first saliva sample (T1) was 4.13 minutes (SD = 4.68). Additionally, the mean amount of time between completing the first and second saliva samples (T1 – T2) was 29.35 minutes (SD = 3.03).

Primary Analyses

Diurnal Cortisol. To assess the hypothesis that the diurnal cortisol rhythm would differ between risky substance users and non-risky substance users, as well as between biological males and females, a 2 (substance use status) × 2 (sex assigned at birth) repeated measures analyses of variance was conducted to assess main effects and interactions on cortisol levels at Time 1, Time 2, Time 3, and Time 4 of collection. Given lack of sufficient power, covariates were not included. Greenhouse-Geiser corrections are reported since the assumption of sphericity was violated, $\chi^2(2) = 15.10$, $p = .01$. The diurnal cortisol curve reflected an expected pattern based on previous literature, with a

significant time effect across the four time points, $F(2.27, 79.49) = 63.46$, $p < .001$, $\eta p^2 = 0.65$. The results revealed that the within-subjects effects of time × sex, $F(2.27, 79.49) = 1.99$, $p = .14$, $\eta p^2 = 0.05$, time × substance use status, $F(2.27, 79.49) = 0.45$, $p = .66$, $\eta p^2 = 0.01$, as well as time × sex × substance use status, $F(2.27, 79.49) = 0.60$, $p = .57$, $\eta p^2 = 0.02$, were not statistically significant. However, after removing the atypical responders from the analysis, the effect sizes increased for time × sex, $F(2.53, 70.78) = 2.60$, $p = .07$, $\eta p^2 = 0.08$, and for time × substance use status, $F(2.53, 70.78) = 1.69$, $p = .18$, $\eta p^2 = 0.06$, but not for time × sex × substance use status, $F(2.53, 70.78) = 0.39$, $p = .73$, $\eta p^2 = 0.01$. See Figures 2 and 3 for the diurnal cortisol slope at all four time points for those assigned male and female at birth and for those displaying low versus moderate to high risk for alcohol-related consequences, respectively.

ACE Exposure and Area Under the Curve. Area under the curve with respect to ground (AUC_G) has been used in previous research examining childhood adversity as an assessment of CAR (Kuras et al., 2017) and was calculated using the first three time points (Pruessner et al., 2003) in order to examine the relationship between ACE score and AUC_G. Specifically, this value was computed using AUC_G = ([T2 + T1]/2 × time) + ([T3 + T2]/2 × time) described by Pruessner and colleagues (2003). After removing atypical responders, results revealed that ACE score did not significantly predict AUC_G, $b = -0.82$, $SE = 0.73$, $\beta = 0.21$, $p = .27$, $R^2 = 0.04$, $F(1, 27) = 1.24$. In examining group differences between those with low (0 to 1) and high (≥2) ACE exposure, results indicated that there was no significant difference in AUC_G between the two groups, $t(27) = 0.94$, $p = .36$, $d = 0.35$. However, there was a significant difference in cortisol levels at T1 (waking) between those with low and high ACE exposure, $t(44) = 3.50$, $p = .001$, $d =$

1.03, with those reporting high ACE exposure exhibiting a blunted waking level of cortisol. See Figure 4 for the diurnal cortisol slope at all four time points for those with low and high ACE exposure.

Discussion

The current program of research illustrates a more nuanced understanding of the relationship among ACEs, perceived and physiological stress, and substance use in a college population. Specifically, the results from Study One align with previous literature on ACEs in an undergraduate population whereby over half of the participants from a large Midwestern university reported having endured at least one ACE (e.g., Grigsby et al., 2020). Furthermore, perceived stress (H1) was higher among those exhibiting high (versus low) risk for alcohol consequences, high (versus low) e-cigarette use, daily (versus never) use of marijuana/THC products, and among females (versus males), which justified hypotheses in Study Two regarding higher cortisol levels among those at moderate to high (versus low) risk for alcohol consequences and among females (versus males).

In partial support of the H2, results of Study One revealed a positive relationship between cumulative exposure to ACEs and consequences of alcohol use and e-cigarette use, which supports previous findings for alcohol (Forster et al., 2019; Grigsby et al., 2020) and e-cigarette (Grigsby et al., 2020) use in a college student population. However, this relationship was not observed between marijuana use and cumulative ACE exposure, which is inconsistent with the college student literature (e.g., Forster et al., 2019). Given these results, perhaps participants who reported using substances in Study One might fall into distinct profiles of use (e.g., by severity or substance[s] of choice) that are

characterized by divergent risk factors that were not examined. For example, one team of researchers identified four profiles of substance use, whereby two patterns were marked by heavy substance use and the other two were marked by varying levels of marijuana use (Sadeh et al., 2021). These researchers found that the recreational marijuana use profile reported lower levels of childhood maltreatment than the profiles characterized by heavy use, and profiles characterized by marijuana use reported using substances to avoid unpleasant emotions and using substances to feel pleasant emotions to a lesser extent than the heavy use profiles. Thus, future research might examine discrepant risk factors associated with different underlying profiles of substance use in college students.

Finally, results of the mediational model (H3) revealed that perceived stress mediated the relationship between ACEs and consequences of alcohol use. Perhaps early life experiences (i.e., of both adversity and resiliency) influence our perceived ability to cope with life stressors or regulate our emotions, which may contribute to maladaptive coping mechanisms such as risky substance use in adolescence and early adulthood. In fact, previous research has revealed that cumulative ACE exposure was predictive of decreased adulthood cognitive flexibility, specifically in participants' tendency to view challenging circumstances as controllable (Kalia & Knauft, 2020). Thus, while exposure to ACEs represents a transdiagnostic risk factor for a myriad of health risk behaviors (e.g., Anda et al., 2006), variables such as perceived stress help to explain substance use outcomes. Future research might examine current life stressors and perceived ability to cope with stress in relation to ACEs and risky substance use.

Study Two examined whether diurnal cortisol rhythm would differ by substance use status and by sex assigned at birth and whether ACE exposure would predict CAR

via AUC_G. First, it should be noted that the current study lacked sufficient power for the analyses that were conducted (i.e., .21 to .36 assuming an alpha level of .05). While the results of the repeated measures ANOVA (H4) were non-significant, there was a medium effect size for the difference in cortisol levels across time between those displaying low versus moderate to high risk for hazardous alcohol use as well as between males and females, whereby those who met criteria for moderate to high risk exhibited heightened cortisol levels across time points compared to those at low risk, and those assigned female at birth displayed higher cortisol levels across time points compared to those assigned male at birth. These findings were consistent with the results of Study One whereby males and those reporting low risk for hazardous drinking reported lower perceived stress levels compared to females and those reporting moderate to high risk for hazardous drinking, respectively. In support of these findings, hyperactivity of the HPA axis has long been recognized in association with risk of substance use (e.g., Kirsch et al., 2020; Lovallo, 2006).

Further, while ACE score did not predict AUC_G (H5), results revealed lower cortisol levels upon waking (T1) among those with high (≥2) ACE exposure compared to those with low (0 to 1) ACEs, which is consistent with previous research (Kuras et al., 2017). Specifically, Kuras and colleagues (2017) also found that, among adults with a mean age of 34, childhood adversity did not predict AUC_G, while the presence of childhood adversity predicted lower waking cortisol levels. Thus, Study Two provides preliminary support for the relationship among ACEs, substance use, and cortisol levels. Given the earlier age of first use for e-cigarettes compared to alcohol and marijuana/THC use in this college student sample, e-cigarette use may represent an important target for

future research examining ACEs and CAR in order to inform trauma- and stress-related awareness and interventions.

Limitations and Future Research

The current studies have several limitations. Conclusions were drawn from a convenience sample of university students who were predominantly white, heterosexual, cisgender, and from households above the poverty level; that said, important information from those of marginalized backgrounds is missing. Moreover, the ACE-Q (Dong et al., 2004) contains questions assessing 10 categories of childhood adversity that do not include items such as bullying, community violence, or interactions with law enforcement or social services. Further, the timing and duration of exposure to ACEs was not assessed but may hold important implications for HPA axis functioning. For example, Weems and Carrion (2007) noted that time since a traumatic event (i.e., recent or distal) had differential impact on mean cortisol level across the day among children with a mean age of 10.7 years. Further, these researchers found that time since the trauma moderated the relationship between cortisol levels and PTSD symptoms. Thus, future research may examine whether particular categories of ACEs with respect to time passed, different ages (e.g., sensitive periods), and durations of exposure may have differential impact on HPA axis functioning and mental health in young adult college students. Similarly, future research may assess current stressors and/or whether childhood adversities continued into adulthood (e.g., still living with household dysfunction) in relation to these variables.

Additionally, participants enrolled with knowledge that each of the studies was assessing childhood adversity, substance use, and other risky behaviors, which may have allowed for selection bias. Further, a number of participants in Study Two reported high

ACE scores but disclosed low or no substance use due to intentional abstinence, cutting back, or religious reasons, which warrants longitudinal assessment of diurnal cortisol rhythm to capture potential physiological fluctuations across individuals' changes in substance use. In addition, data were based on self-report measures of potentially sensitive topics, which may be subject to recall bias, participant bias, or socially desirable responding. Similarly, since saliva samples were completed naturalistically without supervision, participants self-reported their wake time, completion time, and other relevant variables with each sample as a measure of adherence to protocol; while seven participants indicated some level of non-compliance, it is possible that others were non-compliant but did not disclose this. In addition, samples were only collected across one day rather than averaged across two or more days.

Conclusions and Implications

Overall, this study is innovative in that the investigators employed a well-established self-administered cortisol collection method (Saxbe, 2008) that has been previously unexplored in the context of ACEs and risky substance use. The findings provided preliminary support for the relationship among ACEs, substance use, and cortisol levels. Future research in this domain may inform tailored prevention efforts by means of self-collected cortisol samples to identify those at risk for hazardous substance use and other health-compromising behaviors who may have a history of ACEs.

Funding

This research was supported by Psi Chi, the International Honor Society in Psychology, as well as the University of Minnesota Duluth, Department of Psychology.

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Table 1

Prevalence of Each Category of Adverse Childhood Experience and the ACE Score

Adverse Childhood Experience	%
Emotional Abuse	28.5
Parental Divorce or Separation	27.3
Household Mental Illness	25.3
Household Substance Use	20.9
Emotional Neglect	19.0
Physical Abuse	11.1
Incarcerated Household Member	7.5
Sexual Abuse/Molestation	7.1
Mother Treated Violently	4.3
Physical Neglect	4.3
Number of Adverse Childhood Experiences (ACE Score)	
0	44.3
1	19.0
2	13.0
≥3	23.7

Note. Data are from Study One. $N = 253$. The ACE score was determined by summing each reported category of ACE using the ACE-Q (Dong et al., 2004). Range: 0-9.

Table 2

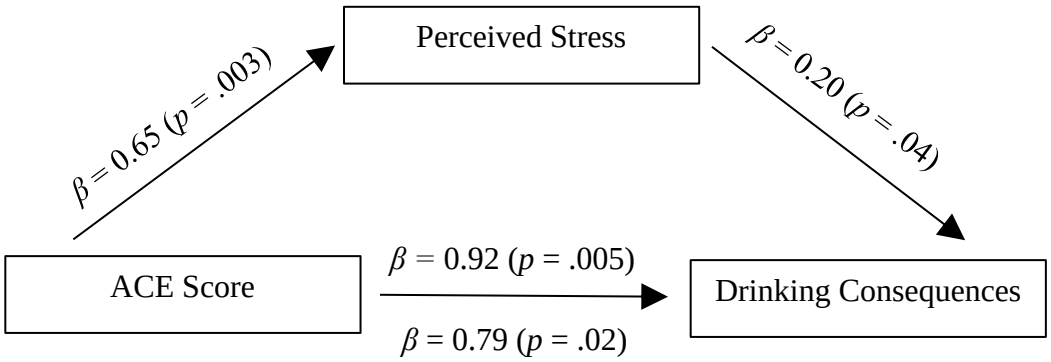
Prevalence of Each Category of Adverse Childhood Experience and the ACE Score

Adverse Childhood Experience	%
Household Mental Illness	46.3
Parental Divorce or Separation	33.3
Emotional Abuse	22.2
Household Substance Use	16.7
Emotional Neglect	11.1
Sexual Abuse/Molestation	11.1
Physical Abuse	9.3
Incarcerated Household Member	9.3
Physical Neglect	5.6
Mother Treated Violently	3.7
Number of Adverse Childhood Experiences (ACE Score)	
0	35.2
1	14.8
2	16.7
≥3	33.3

Note. Data are from Study Two. $N = 54$. The ACE score was determined by summing each reported category of ACE using the ACE-Q (Dong et al., 2004). Range: 0-9.

Figure 1

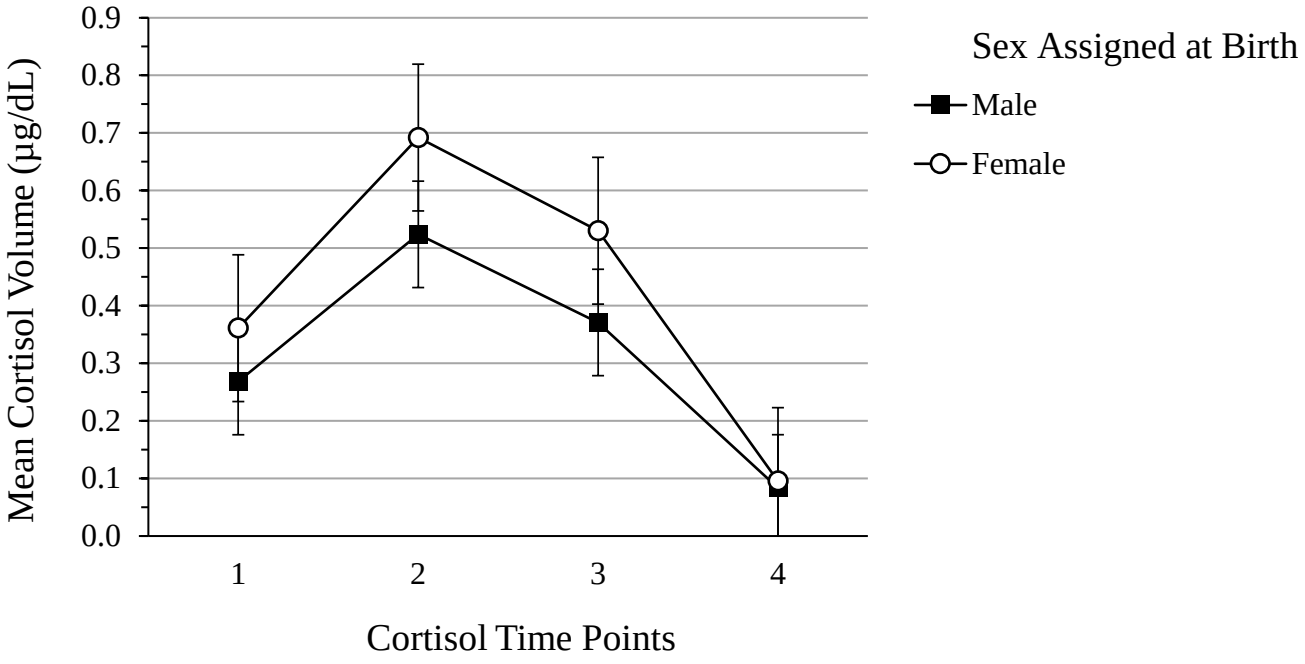
ACE Score as a Predictor of Drinking Consequences Through Perceived Stress



Note. Indirect effect = 0.13, *SE* = 0.07, 95% CI = [0.01, 0.28]. ACE score was computed using the ACE-Q (Dong et al., 2004). Consequences of drinking were computed using the YAACQ (Read et al., 2006). Perceived stress was computed using the PSS (Cohen et al., 1983).

Figure 2

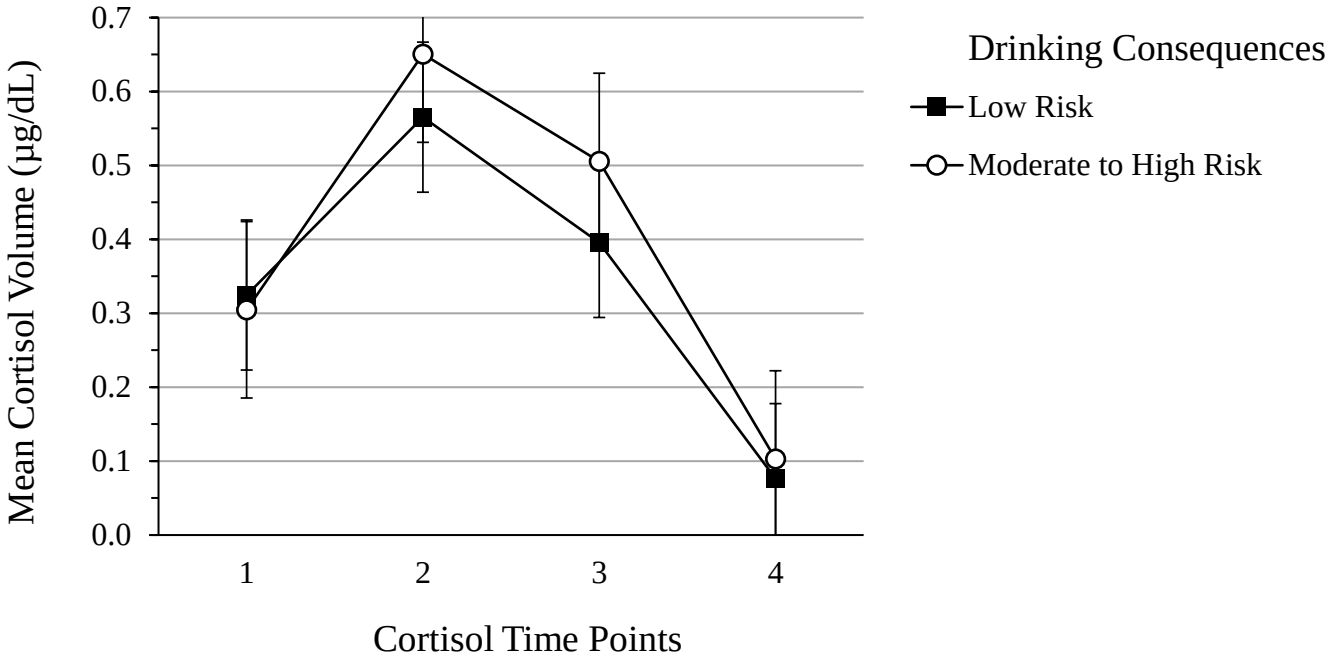
Diurnal Cortisol Rhythm by Sex Assigned at Birth



Note. N = 32 (14 males and 18 females). A medium effect of time × sex was revealed, $F(2.53, 70.78) = 2.60$, $p = .07$, $\eta p^2 = 0.09$. T1 represents samples taken immediately upon awakening, T2 represents samples taken 30 minutes post-awakening, T3 represents samples taken 60 minutes post-awakening, and T4 represents samples taken around 8:00 PM.

Figure 3

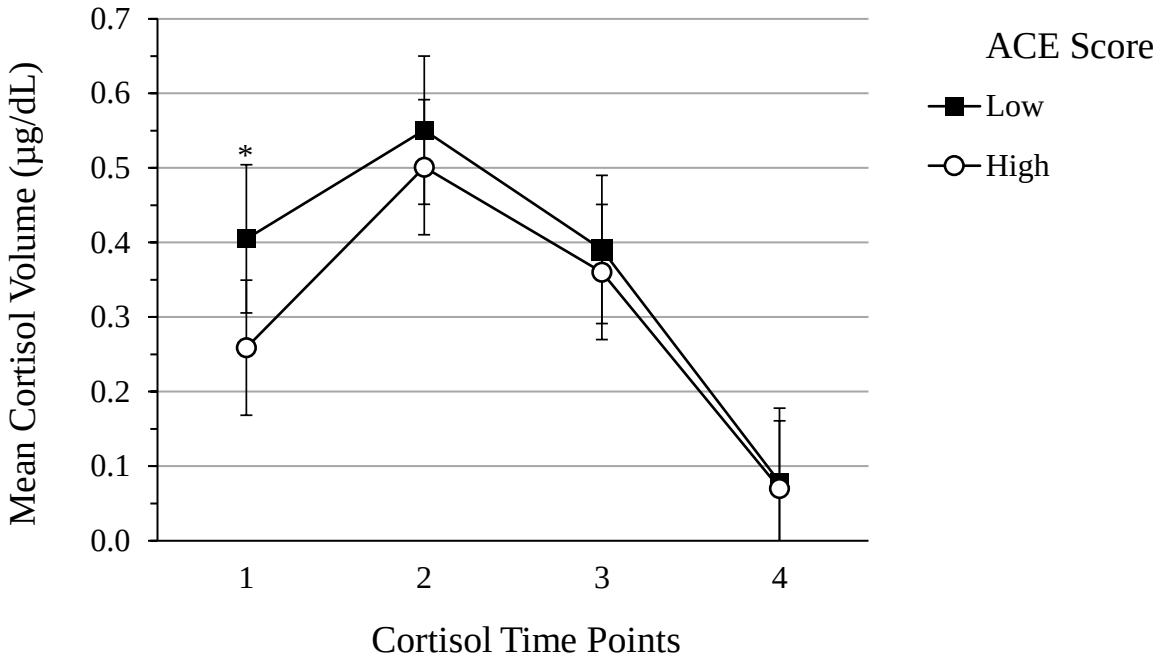
Diurnal Cortisol Rhythm by Risky Drinking Status



Note. N = 32 (9 met criteria for moderate to high risk for hazardous drinking in the past year while 23 were at low risk for hazardous drinking using established cutoff scores in accordance with the YAACQ [Read et al., 2006; 2016]). A medium effect of time × substance use status was found, $F(2.53, 70.78) = 1.69$, $p = .18$, $\eta p^2 = 0.06$. T1 represents samples taken immediately upon awakening, T2 represents samples taken 30 minutes post-awakening, T3 represents samples taken 60 minutes post-awakening, and T4 represents samples taken around 8:00 PM.

Figure 4

Diurnal Cortisol Rhythm by High and Low ACE Score



Note. $N = 46$ (23 with low [0 to 1] ACE exposure and 23 with high [≥2] ACE exposure). T1 represents samples taken immediately upon awakening, T2 represents samples taken 30 minutes post-awakening, T3 represents samples taken 60 minutes post-awakening, and T4 represents samples taken around 8:00 PM.

*Significant at $p = .001$.

Appendix A

Study One SONA Recruitment Message

The purpose of the proposed study is to assess substance use behaviors, positive and negative life events, and perceived stress in college-aged adults. The surveys will be completed entirely online. Participants will receive one course credit for completing this study. Participants who meet study eligibility criteria will be invited to participate in the laboratory session. Eligibility criteria include: 1) Participants must be at least 18 years old; 2) Participants must have been speaking English for at least 10 years.

Appendix B

Study Two SONA Recruitment Message

The purpose of this study is to examine the relationship among early life events, substance use behaviors, and hormones in saliva. The study involves a screening and laboratory visit as well as collecting four saliva samples within your residence. During the screening visit, participants will complete a series of questionnaires to determine study eligibility. Participants will receive either a $15 ClinCard OR two course credits for completing all study protocol. Participants who meet study eligibility criteria will be invited to participate in the laboratory session. Eligibility criteria include: 1) Participants must be at least 18 years old; 2) Participants must have been speaking English for at least 10 years.

Appendix C

Study Two Recruitment Flyer

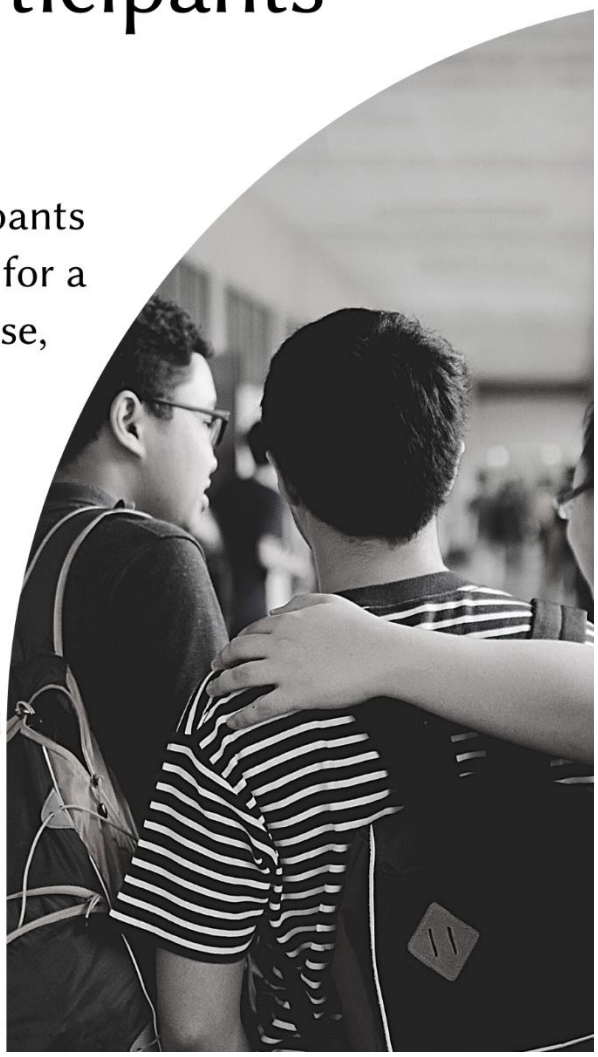
University of Minnesota Duluth
DEPARTMENT OF PSYCHOLOGY

Spit for Science!
Research Participants Needed

UMD is recruiting participants **ages 18 years and older** for a study on how substance use, early life events, and hormones in saliva are related.

The study involves an in-person screening, an online survey, and four saliva samples that you can take within your residence. Participants will receive **two SONA credits** OR a **$15 ClinCard** for completing all study protocol.

Please email our study team at **nclabumd@d.umn.edu** for more information.



Appendix D

The Adverse Childhood Experiences Questionnaire (ACE-Q; Dong et al., 2004)

While you were growing up, during your first 18 years of life:

1. Did a parent or other adult in the household often …
   Swear at you, insult you, put you down, or humiliate you?
   or
   Act in a way that made you afraid that you might be physically hurt?
   Yes / No
2. Did a parent or other adult in the household often …
   Push, grab, slap, or throw something at you?
   or
   Ever hit you so hard that you had marks or were injured?
   Yes / No
3. Did an adult or person at least 5 years older than you ever…
   Touch or fondle you or have you touch their body in a sexual way?
   or
   Try to or actually have oral, anal, or vaginal sex with you?
   Yes / No
4. Did you often feel that …
   No one in your family loved you or thought you were important or special?
   or
   Your family didn't look out for each other, feel close to each other, or support each other?
   Yes / No
5. Did you often feel that …
   You didn't have enough to eat, had to wear dirty clothes, and had no one to protect you?
   or
   Your parents were too drunk or high to take care of you or take you to the doctor if you needed it?
   Yes / No
6. Were your parents ever separated or divorced?
   Yes / No
7. Was your mother or stepmother:
   Often pushed, grabbed, slapped, or had something thrown at her?
   or
   Sometimes or often kicked, bitten, hit with a fist, or hit with something hard?
   or
   Ever repeatedly hit over at least a few minutes or threatened with a gun or knife?
   Yes / No

8. Did you live with anyone who was a problem drinker or alcoholic or who used street drugs?

Yes / No

9. Was a household member depressed or mentally ill or did a household member attempt suicide?

Yes / No

10. Did a household member go to prison?

Yes / No

Appendix E

The Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001)

1. How often do you have a drink containing alcohol?
    (0) Never [Skip to Qs 9-10]
    (1) Monthly or less
    (2) 2 to 4 times a month
    (3) 2 to 3 times a week
    (4) 4 or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?
    (0) 1 or 2
    (1) 3 or 4
    (2) 5 or 6
    (3) 7, 8, or 9
    (4) 10 or more
3. How often do you have six or more drinks on one occasion?
    (0) Never
    (1) Less than monthly
    (2) Monthly
    (3) Weekly
    (4) Daily or almost daily

Skip to Questions 9 and 10 if Total Score for Questions 2 and 3 = 0

4. How often during the last year have you found that you were not able to stop drinking once you had started?
    (0) Never
    (1) Less than monthly
    (2) Monthly
    (3) Weekly
    (4) Daily or almost daily
5. How often during the last year have you failed to do what was normally expected from you because of drinking?
    (0) Never
    (1) Less than monthly
    (2) Monthly
    (3) Weekly
    (4) Daily or almost daily
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
    (0) Never
    (1) Less than monthly
    (2) Monthly

(3) Weekly
(4) Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remorse after drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily
9. Have you or someone else been injured as a result of your drinking?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year
10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year

Appendix F

The Young Adult Alcohol Consequences Questionnaire (YAACQ; Read et al., 2006)

In the past year…	0	1
1. While drinking, I have done or said embarrassing things.	No	Yes
2. The quality of my work or schoolwork has suffered because of my drinking.	No	Yes
3. I have felt badly about myself because of my drinking.	No	Yes
4. I have driven a car when I knew I had too much to drink to drive safely.	No	Yes
5. I have had a hangover (headache, sick stomach) the morning after I had been drinking.	No	Yes
6. I have spent too much money on alcohol.	No	Yes
7. I have showed up late to work or school because of drinking, a hangover, or illness caused by drinking.	No	Yes
8. I have passed out from drinking.	No	Yes
9. I have taken foolish risks when I have been drinking.	No	Yes
10. I have felt very sick to my stomach or thrown up after drinking.	No	Yes
11. I have gotten into trouble at work or school because of drinking.	No	Yes
12. My drinking has caused serious, recurrent physical problems, or made an existing physical problem worse.	No	Yes
13. I often drank more than I originally had planned.	No	Yes
14. My drinking has created problems between myself and my boyfriend/girlfriend/spouse, parents, or other near relatives.	No	Yes
15. I have been unhappy because of my drinking.	No	Yes
16. I have been fired from a job, or suspended or expelled from school because of drinking.	No	Yes
17. I have gotten into physical fights because of drinking.	No	Yes

18. I have been told by a doctor that drinking was harming my health.	No	Yes
19. I have spent too much time drinking.	No	Yes
20. I have not gone to work or missed classes at school because of drinking, a hangover, or illness caused by drinking.	No	Yes
21. I have felt like I needed a drink after I'd gotten up (that is, before breakfast).	No	Yes
22. I have become very rude, obnoxious or insulting after drinking.	No	Yes
23. I have felt guilty about my drinking.	No	Yes
24. I have damaged property, or done something disruptive such as setting off a false fire alarm, or other things like that after I had been drinking.	No	Yes
25. Because of my drinking, I have not eaten properly.	No	Yes
26. I have been less physically active because of drinking.	No	Yes
27. I have had "the shakes" after stopping or cutting down on drinking (e.g., hands shake so that coffee cup rattles in the saucer or have trouble lighting a cigarette).	No	Yes
28. My boyfriend/girlfriend/spouse/parents have complained to me about my drinking.	No	Yes
29. I have woken up in an unexpected place after heavy drinking.	No	Yes
30. I have been less involved in extra-curricular campus activities because of my drinking.	No	Yes
31. I have found that I needed larger amounts of alcohol to feel any effect, or that I could no longer get high or drunk on the amount that used to get me high or drunk.	No	Yes
32. As a result of drinking, I neglected to protect myself or my partner from a sexually transmitted disease (STD) or an unwanted pregnancy.	No	Yes
33. I have spent too much money while drinking.	No	Yes
34. I have felt that I needed alcohol, or was dependent on alcohol.	No	Yes
35. I have lost friends (including boyfriends or girlfriends) because of my drinking.	No	Yes

36. I have neglected my obligations to family, work, or school because of drinking.	No	Yes
37. I often have ended up drinking on nights when I had planned not to drink.	No	Yes
38. When drinking, I have done impulsive things that I regretted later.	No	Yes
39. I have often found it difficult to limit how much I drink.	No	Yes
40. My drinking has gotten me into sexual situations I later regretted.	No	Yes
41. I've not been able to remember large stretches of time while drinking heavily.	No	Yes
42. While drinking, I have said harsh or cruel things to someone.	No	Yes
43. Because of my drinking I have not slept properly.	No	Yes
44. My physical appearance has been harmed by my drinking.	No	Yes
45. I have said things while drinking that I later regretted.	No	Yes
46. I have spent a great deal of time drinking, or recovering from drinking.	No	Yes
47. My physical health has been harmed by drinking.	No	Yes
48. I have awakened the day after drinking and found that I could not remember a part of the evening before.	No	Yes
49. I have been overweight because of drinking.	No	Yes
50. I have been arrested for driving under the influence of alcohol, or for other drunken behavior.	No	Yes
51. I haven't been as sharp mentally because of my drinking.	No	Yes
52. I have received a lower grade on an exam or paper than I ordinarily could have because of my drinking.	No	Yes
53. I have tried to quit drinking because I thought I was drinking too much.	No	Yes
54. I have felt anxious, agitated, or restless after stopping or cutting down on drinking.	No	Yes
55. My drinking has prevented me from taking full advantage of the college experience.	No	Yes

56. I have had serious emotional problems because of drinking.	No	Yes
57. My drinking has damaged my social life, popularity, or reputation.	No	Yes
58. I have not had as much time to pursue activities or recreation because of drinking.	No	Yes
59. While drinking, I have been a victim of theft or robbery.	No	Yes
60. I have sought help or treatment for my drinking (e.g., counseling, AA, friend, clergy, family, self-help book, etc.)	No	Yes
61. I have injured someone else while drinking or intoxicated.	No	Yes
62. I have often thought about needing to cut down or stop drinking.	No	Yes
63. I was a victim of physical or sexual assault while I was intoxicated.	No	Yes
64. I have had less energy or felt tired because of my drinking.	No	Yes
65. I have had an accident while drinking or intoxicated.	No	Yes
66. I have had a blackout after drinking heavily (i.e., could not remember hours at a time).	No	Yes
67. Drinking has made me feel depressed or sad.	No	Yes

Appendix G

Daily Drinking Questionnaire (DDQ; Collins et al., 1985)

STANDARD DRINK CONVERSION

When asked how much you drink in the following questions use this chart.

ONE STANDARD DRINK IS EQUAL TO:

Standard American BEER (3-5% alcohol) — 12 oz. Can, Bottle or Glass

Microbrew or European BEER (8%-12% alcohol) — 1/2 of a 12 oz. Can or Bottle

WINE (12 – 17% alcohol) — 4 oz. Glass

WINE Cooler — 10 oz. Bottle

HARD LIQUOR (80-proof, 40% alcohol) — 1-1/2 oz. or One Standard Shot

HARD LIQUOR (100-proof, 50% alcohol) — 1 oz.

WINE: 1 Bottle

25 oz. (12 – 17% alcohol) = 5 standard drinks
40 oz. (12 – 17% alcohol) = 8 standard drinks

HARD LIQUOR: 1 Bottle

12 oz. = 8 standard drinks
25 oz. = 17 standard drinks
40 oz. = 27 standard drinks

DDQ-R (Daily Drinking Questionnaire-Revised)

Gender: Male_____ Female_____ **Height** _____' _____" (Feet) (Inches) **Weight**_____ lbs.

INSTRUCTIONS FOR RECORDING DRINKING DURING A TYPICAL WEEK

IN THE CALENDAR BELOW, PLEASE FILL-IN YOUR DRINKING RATE AND TIME DRINKING DURING A **TYPICAL WEEK** IN THE LAST **30 DAYS.**

First, think of a *typical week* in the last *30 days you.* (Where did you live? What were your regular weekly activities? Where you working or going to school? Etc.) Try to remember as accurately as you can, *how much* and for *how long* you *typically drank* in a week during that one month period?

For each day of the week in the calendar below, fill in the **number of standard drinks typically consumed on that day** in the upper box and the **typical number of hours you drank** that day in the lower box.

Day of Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Number of *Drinks*							
Number of *Hours Drinking*							

INSTRUCTIONS FOR RECORDING DRINKING FOR YOUR HEAVIEST DRINKING WEEK

IN THE CALENDAR BELOW, PLEASE FILL-IN YOUR DRINKING RATE AND TIME DRINKING DURING YOUR **HEAVIEST DRINKING WEEK** IN THE LAST **30 DAYS.**

First, think of your *heaviest drinking week* in the last *30 days*. (Where did you live? What were your regular weekly activities? Where you working or going to school? Etc.)
Try to remember as accurately as you can, *how much* and for *how long* did you drink during your *heaviest drinking week* in that one month period?

For each day of the week in the calendar below, fill in the **number of standard drinks consumed on that day** in the upper box and the **number of hours you drank** that day in the lower box.

Day of Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Number of *Drinks*							
Number of *Hours Drinking*							

Drinking Quantity/Frequency Index (Cahallan's Q/F Index)

1. **How often did you drink during the last *month*?** (check one)

   a. I did not drink at all.
   b. About once a month.
   c. Two to three times a month.
   d. Once or twice a week.
   e. Three to four times a week.
   f. Nearly every day.
   g. Once a day or more.

2. **Think of a typical weekend evening** (Friday or Saturday) during the last *month*.. How **much** did you drink on that evening? (check one)

0 drinks	8 drinks	16 drinks	24 drinks
1 drinks	9 drinks	17 drinks	25 drinks
2 drinks	10 drinks	18 drinks	26 drinks
3 drinks	11 drinks	19 drinks	27 drinks
4 drinks	12 drinks	20 drinks	28 drinks
5 drinks	13 drinks	21 drinks	29 drinks
6 drinks	14 drinks	22 drinks	30 drinks
7 drinks	15 drinks	23 drinks	*More than* 30

3. **Think of the occasion** (any day of the week) **you drank the most** during the last *month*. How **much** did you drink? (check one)

0 drinks	8 drinks	16 drinks	24 drinks
1 drinks	9 drinks	17 drinks	25 drinks
2 drinks	10 drinks	18 drinks	26 drinks
3 drinks	11 drinks	19 drinks	27 drinks
4 drinks	12 drinks	20 drinks	28 drinks
5 drinks	13 drinks	21 drinks	29 drinks
6 drinks	14 drinks	22 drinks	30 drinks
7 drinks	15 drinks	23 drinks	*More than* 30

Appendix H

The Marijuana Problem Inventory (MPI; Modified; Vandrey et al., 2005)

Different things happen to people when they are using marijuana or using other products containing THC (e.g., edibles, vapes, tinctures). Some of these things are listed below. Read each statement carefully and select the answer that best describes your reactions.

How many times did the following things happen to you while you were using marijuana/THC or because of your marijuana/THC use during the last year?

1. Not able to do your homework or study for a test
   - Never
   - 1-2 times
   - 3-5 times
   - 6-10 times
   - More than 10 times
2. Got into fights, acted bad, or did mean things
   - Never
   - 1-2 times
   - 3-5 times
   - 6-10 times
   - More than 10 times
3. Missed out on other things because you spent too much money on marijuana/THC products
   - Never
   - 1-2 times
   - 3-5 times
   - 6-10 times
   - More than 10 times
4. Went to work or school high or stoned
   - Never
   - 1-2 times
   - 3-5 times
   - 6-10 times
   - More than 10 times
5. Caused shame or embarrassment to someone
   - Never
   - 1-2 times
   - 3-5 times
   - 6-10 times
   - More than 10 times
6. Neglected your responsibilities
   - Never
   - 1-2 times

3-5 times
6-10 times
More than 10 times

7. Relatives avoided you
   Never
   1-2 times
   3-5 times
   6-10 times
   More than 10 times
8. Felt that you needed more marijuana/THC than you used to use in order to get the same effect
   Never
   1-2 times
   3-5 times
   6-10 times
   More than 10 times
9. Tried to control your marijuana/THC use by trying to smoke marijuana only certain times of day or certain places
   Never
   1-2 times
   3-5 times
   6-10 times
   More than 10 times
10. Had withdrawal symptoms, that is, felt sick because you stopped or cut down on smoking marijuana or using other THC products
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
11. Noticed a change in your personality
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
12. Felt that you had a problem with school
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
13. Missed a day (or part of a day) of school or work

Never
1-2 times
3-5 times
6-10 times
More than 10 times

14. Tried to cut down on smoking marijuana/using THC products
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
15. Suddenly found yourself in a place that you could not remember getting to
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
16. Passed out or fainted suddenly
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
17. Had a fight, argument, or bad feelings with a friend
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
18. Had a fight, argument or bad feelings with a family member
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
19. Kept smoking marijuana/using THC when you promised yourself not to
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
20. Felt you were going crazy
    Never

1-2 times
3-5 times
6-10 times
More than 10 times

21. Had a bad time
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
22. Felt physically or physiologically dependent on marijuana/THC
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
23. Was told by a friend or neighbor to stop or cut down your marijuana/THC use
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
24. Felt paranoid or overly nervous in everyday life
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
25. Felt unmotivated to do things you needed to do in your everyday life
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
26. Lost interest in things you once enjoyed
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
27. Noticed that your memory was not as good as it used to be
    Never
    1-2 times

3-5 times
6-10 times
More than 10 times

28. Lost some physical coordination in everyday activities
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times
29. Had trouble thinking clearly in everyday activities
    Never
    1-2 times
    3-5 times
    6-10 times
    More than 10 times

Appendix I

The Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991)

1. How soon after you wake up do you smoke your first cigarette?
    (3) Within 5 minutes
    (2) 6-30 minutes
    (1) 31-60 minutes
    (0) After 60 minutes
2. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g. in church, at the library, in a cinema, etc.)?
    (1) Yes
    (0) No
3. Which cigarette would you hate most to give up?
    (1) The first one in the morning
    (0) All others
4. How many cigarettes/day do you smoke?
    (0) 10 or less
    (1) 11-20
    (2) 21-30
    (3) 31 or more
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?
    (1) Yes
    (0) No
6. Do you smoke if you are so ill that you are in bed most of the day?
    (1) Yes
    (0) No

The Fagerstrom Test for Nicotine Dependence for e-Cigarettes (e-FTND; Etter & Eissenberg, 2015)

1. How soon after you wake up do you smoke your first e-cigarette?
    (3) Within 5 minutes
    (2) 6-30 minutes
    (1) 31-60 minutes
    (0) After 60 minutes
2. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g. in church, at the library, in a cinema, etc.)?
    (1) Yes
    (0) No
3. Which e-cigarette would you hate most to give up?
    (1) The first one in the morning
    (0) All others
4. How many times per day do you smoke e-cigarettes?
    *Continuous scale 1-50
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?
    (1) Yes
    (0) No
6. Do you smoke if you are so ill that you are in bed most of the day?
    (1) Yes
    (0) No

The Fagerstrom Test for Nicotine Dependence for Smokeless Tobacco (FTND-ST; Ebbert et al., 2006)

1. How soon after you wake up do you place your first dip?
    (3) Within 5 minutes
    (2) 6-30 minutes
    (1) 31-60 minutes
    (0) After 60 minutes
2. How often do you intentionally swallow tobacco juice?
    (2) Always
    (1) Sometimes
    (0) Never
3. Which chew would you hate most to give up?
    (1) The first one in the morning
    (0) All others
4. How many cans/pouches per week do you use?
    (2) More than 3
    (1) 2-3
    (0) 1
5. Do you chew more frequently during the first hours after waking than during the rest of the day?
    (1) Yes
    (0) No
6. Do you chew if you are so ill that you are in bed most of the day?
    (1) Yes
    (0) No

Appendix J

Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977)

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

	During the Past Week			
	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me.	0	1	2	3
2. I did not feel like eating; my appetite was poor.	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family or friends.	0	1	2	3
4. I felt I was just as good as other people.	0	1	2	3
5. I had trouble keeping my mind on what I was doing.	0	1	2	3
6. I felt depressed.	0	1	2	3
7. I felt that everything I did was an effort.	0	1	2	3
8. I felt hopeful about the future.	0	1	2	3
9. I thought my life had been a failure.	0	1	2	3
10. I felt fearful.	0	1	2	3
11. My sleep was restless.	0	1	2	3
12. I was happy.	0	1	2	3
13. I talked less than usual.	0	1	2	3
14. I felt lonely.	0	1	2	3

15. People were unfriendly.	0	1	2	3
16. I enjoyed life.	0	1	2	3
17. I had crying spells.	0	1	2	3
18. I felt sad.	0	1	2	3
19. I felt that people dislike me.	0	1	2	3
20. I could not get “going.”	0	1	2	3

Appendix K

UCLA Loneliness Scale (Russell et al., 1980)

Scale:
INSTRUCTIONS: Indicate how often each of the statements below is descriptive of you.

Statement	Never	Rarely	Sometimes	Often
*1. How often do you feel that you are "in tune" with the people around you?	1	2	3	4
2. How often do you feel that you lack companionship?	1	2	3	4
3. How often do you feel that there is no one you can turn to?	1	2	3	4
4 How often do you feel alone?	1	2	3	4
*5. How often do you feel part of a group of friends?	1	2	3	4
*6. How often do you feel that you have a lot in common with the people around you?	1	2	3	4
7. How often do you feel that you are no longer close to anyone?	1	2	3	4
8. How often do you feel that your interests and ideas are not shared by those around you?	1	2	3	4
*9. How often do you feel outgoing and friendly?	1	2	3	4
*10. How often do you feel close to people?	1	2	3	4
11. How often do you feel left out?	1	2	3	4
12. How often do you feel that your relationships with others are not meaningful?	1	2	3	4
13. How often do you feel that no one really knows you we11?	1	2	3	4
14. How often do you feel isolated from others?	1	2	3	4
*15. How often do you fee1 you can find companionship when you want it?	1	2	3	4
*16. How often do you feel that there are people who really understand you?	1	2	3	4
17, How often do you feel shy?	1	2	3	4
18. How often do you feel that people are around you but not with you?	1	2	3	4
*19. How often do you feel that there are people you can talk to?	1	2	3	4
*20. How often do you feel that there are people you can turn to?	1	2	3	4

Scoring:
The items with an asterisk are reverse scored. Keep scoring on a continuous basis.

Appendix L

The Risky, Impulsive, and Self-Destructive Behavior Questionnaire (RISQ; Sadeh & Baskin-Sommers, 2016)

For each behavior, fill-in how many times you did it in your lifetime (A) & the total number of times you did it the past month (B). ***Enter one number for each time period, even if it is your best guess***. ***Please do not put a range, but enter a single number*** (e.g., behaviors engaged in everyday for multiple years can be written in as 1000+, behaviors engaged in daily for a single year can be written in as 365, any other frequency should be estimated using your best guess). If you have ever done the behavior, write how old you were the first time (C) and check the box if the behavior ever caused you any problems, regardless of the specific problem (D). For the last two columns (E & F), use the scale in the box to rate how much you agree with each statement from 0 = Strongly Disagree to 4 = Strongly Agree. ***Please provide ratings for both statements (E & F), and treat them as separate questions***.

	A **How many times** total have you done this **in your life**?	B **How many times** have you done this **in the past month**?	C **How old** were you the **first time**?	D Did it ever **cause you any problems**, such as • going to the hospital • legal trouble • problems at work, with family or friends		E I do this behavior to **stop feeling upset, distressed, or overwhelmed**					F I do this behavior to **feel excitement, to get a thrill, or to feel pleasure**				
	# TOTAL	# Past MONTH	Age	No	Yes	Strongly Disagree	Somewhat Agree	Equally Disagree/Agree	Somewhat Agree	Strongly Agree	Strongly Disagree	Somewhat Agree	Equally Disagree/Agree	Somewhat Agree	Strongly Agree
Shoplifted things				○	○	○	○	○	○	○	○	○	○	○	○
Drove 30mph or faster over the speed limit				○	○	○	○	○	○	○	○	○	○	○	○
Bet on sports, horses, or other animals				○	○	○	○	○	○	○	○	○	○	○	○
Used crack or cocaine				○	○	○	○	○	○	○	○	○	○	○	○
Bought drugs				○	○	○	○	○	○	○	○	○	○	○	○
Impulsively bought stuff you did not need & won't use				○	○	○	○	○	○	○	○	○	○	○	○
Had unprotected sex with someone you just met or didn't know well				○	○	○	○	○	○	○	○	○	○	○	○
Gotten into a physical fight				○	○	○	○	○	○	○	○	○	○	○	○
Had sex for money or drugs				○	○	○	○	○	○	○	○	○	○	○	○
Drank alcohol or drugs until you blacked or passed out				○	○	○	○	○	○	○	○	○	○	○	○
Used hallucinogens, LSD, or mushrooms				○	○	○	○	○	○	○	○	○	○	○	○
Gone to work/school intoxicated or high				○	○	○	○	○	○	○	○	○	○	○	○

Attacked someone with a weapon, such as a knife or gun				○	○	○	○	○	○	○	○	○	○	○	○	○
Punched or hit someone with a fist or object				○	○	○	○	○	○	○	○	○	○	○	○	○
Cut, burned, or hurt yourself on purpose without trying to die				○	○	○	○	○	○	○	○	○	○	○	○	○
Lost more money than you could afford gambling				○	○	○	○	○	○	○	○	○	○	○	○	○
Threatened to physically hurt someone				○	○	○	○	○	○	○	○	○	○	○	○	○
Threatened someone with a weapon, such as a knife or gun				○	○	○	○	○	○	○	○	○	○	○	○	○
Used heroin				○	○	○	○	○	○	○	○	○	○	○	○	○
Destroyed or vandalized property				○	○	○	○	○	○	○	○	○	○	○	○	○
Drank 5 or more alcoholic drinks in 3 hours or less				○	○	○	○	○	○	○	○	○	○	○	○	○
Paid for sex				○	○	○	○	○	○	○	○	○	○	○	○	○
Sold drugs				○	○	○	○	○	○	○	○	○	○	○	○	○
Robbed someone				○	○	○	○	○	○	○	○	○	○	○	○	○
Used marijuana				○	○	○	○	○	○	○	○	○	○	○	○	○
Had difficulty stopping eating				○	○	○	○	○	○	○	○	○	○	○	○	○
Been in 2 or more sexual relationships at the same time				○	○	○	○	○	○	○	○	○	○	○	○	○
Bought expensive items you could not afford on the spur of the moment				○	○	○	○	○	○	○	○	○	○	○	○	○
Abused multiple drugs at once				○	○	○	○	○	○	○	○	○	○	○	○	○
Played lotteries, card games for money, or went to the casino				○	○	○	○	○	○	○	○	○	○	○	○	○
Gambled illegally (not part of a legal business, using a bookie)				○	○	○	○	○	○	○	○	○	○	○	○	○
Abused prescription medication				○	○	○	○	○	○	○	○	○	○	○	○	○
Ate a lot of food when not hungry				○	○	○	○	○	○	○	○	○	○	○	○	○
Ran red lights or ignored stop signs				○	○	○	○	○	○	○	○	○	○	○	○	○
Stole money				○	○	○	○	○	○	○	○	○	○	○	○	○

Appendix M

Life Events Questionnaire (LEQ; Norbeck, 1984; Sarason et al., 1978)

Listed below are a number of events, which may bring about changes in the lives of those who experience them.

Indicate which of the following events that have occurred in your life during the past year and indicate whether these events were Good or Bad.

Show how much the event affected your life by checking no effect, some effect, moderate effect, or great effect.

During the past year, have you experienced:

1. Major personal illness or injury
2. Major change in eating habits
3. Major change in sleeping habits
4. Major change in usual type and/or amount of recreation
5. Major dental work
6. Pregnancy
7. Miscarriage or abortion
8. Major difficulties with birth control pills or devices
9. Difficulty finding a job
10. Beginning work outside the home
11. Changing to a new type of work
12. Changing your work hours or conditions
13. Change in your responsibilities at work
14. Troubles at work with your employer or co-workers
15. Troubles at work with your employer or co-workers
16. Being fired or laid off from work
17. Beginning or ceasing school, college, or training program
18. Change of school, college, or training program
19. Change in career goal or academic major
20. Problems in school, college, or training program
21. Difficulty finding housing
22. Changing residence within the same town or city
23. Moving to a different town, city, state, or country
24. Major change in your life conditions (home improvements or a decline in your home or neighborhood
25. Began a new, close, personal relationship
26. Became engaged
27. Problems with significant other
28. Breaking up with a partner or breaking an engagement
29. Girlfriend's pregnancy
30. Girlfriend having a miscarriage or abortion

31. Beginning to live with someone
32. Beginning to live with someone
33. Infidelity
34. Major change in the health or behavior of a family member or close friend (illness, accidents, drug or disciplinary problems, etc.)
35. Death of a family member or close friend
36. Change in marital status of your parents
37. Major personal achievement
38. Major decision regarding your immediate future
39. Change in your personal habits (your dress, life-style, hobbies, etc.)
40. Change in your religious beliefs
41. Change in your political beliefs
42. Loss or damage of personal property
43. Took a vacation
44. Took a trip other than a vacation
45. Change in family get-togethers
46. Change in your social activities (clubs, movies, visiting)
47. Made new friends
48. Broke up with a friend
49. Acquired or lost a pet
50. Major change in finances (increased or decreased income)
51. Took on a moderate purchase, such as TV, car, freezer, etc.
52. Took on a major purchase or loan, such as a home, business, property, etc.
53. Being robbed or a victim of identity theft
54. Being a victim of a violent act (rape, assault, etc.)
55. Involved in an accident
56. Involved in a law suit
57. Involved in a minor violation of the law (traffic tickets, disturbing the peace, etc.)
58. Please list any other recent experiences that have had an impact on your life: _____

Participants endorsed *yes* or *no* for having experienced the above events. Following each statement, participants were asked:

What type of effect did this event have?

Good
Bad

What effect did this event have on your life?

No effect
Some effect
Moderate effect
Great effect

Appendix N

Demographics

Please provide a response for the following questions. All information provided will be kept confidential and will be used only for the purposes of this study.

Age (in years): _____

What is your sex assigned at birth?
- Male
- Female
- Intersex
- I would prefer not to disclose
- A sex not listed (please specify): _____

What gender do you identify with? (Check all that apply)
- Man
- Woman
- Transgender man
- Transgender woman
- Non-binary/gender queer
- I would prefer not to disclose
- A gender not listed (please specify): _____

Which of the following best describes your sexual identity? (Check all that apply)
- Heterosexual (Straight)
- Gay
- Lesbian
- Bisexual
- Queer
- Pansexual
- Asexual
- Unlabeled
- Don't know
- A sexual identity not listed (please specify): _____

What is your family or household's income level?
- Less than $10,000
- $10,000-$20,000
- $20,000 - $55,000
- $55,000-$75,000
- $75,000-$100,000
- $100,000+
- Don't know or prefer not to answer

Which of the following best describes your race/ethnicity? (Check all that apply)
- White
- Black or African American

American Indian or Alaska Native
Asian
Native Hawaiian or Pacific Islander
Hispanic/Latinx
From multiple races
A race not listed (please specify): _____

What is your height (in inches)? _____

What is your weight (in pounds)? _____

What is your class status?
Freshman
Sophomore
Junior
Senior
Graduate student
A class not listed (Please specify): _____

What type of housing do you have?
Dorms/on-campus housing
Off-campus housing
A type of housing not listed (Please specify): _____

What is your current college GPA (on a 4.0 scale)? _____

Do you use oral contraceptives (e.g., birth control pills)? (For those who selected that their sex assigned at birth is not male)
No
Yes

How many days ago was your last menstrual cycle? (For those who selected that their sex assigned at birth is not male)
No
Yes

At what age did you first try drinking alcohol? (More than a few sips) _____

Do you currently smoke marijuana?
No, I have never smoked marijuana
No, but I have in the past
Yes, occasionally (not daily)
Yes, I smoke marijuana every day

Do you currently use any products containing THC (e.g., edibles, vapes, tinctures)?
No, I have never used THC products
No, but I have in the past
Yes, occasionally (not daily)
Yes, I use THC products every day

Are you currently using an electronic cigarette (e-cigarette)?
No, I have never used one

No, but I have in the past
Yes, occasionally (not daily)
Yes, I use one every day

Compared to a regular (traditional) cigarette, how harmful do you think e-cigarettes are?
Less harmful
Equally as harmful
More harmful

At what age did you first try an e-cigarette? _____

Do you currently smoke traditional cigarettes?
No, I have never smoked cigarettes
No, but I have smoked cigarettes in the past
Yes, occasionally (not daily)
Yes, I smoke cigarettes every day

At what age did you first try a cigarette? _____

Do you currently use smokeless tobacco (e.g., chewing tobacco, snuff, dissolvables)?
No, I have never used smokeless tobacco
No, but I have used smokeless tobacco in the past
Yes, occasionally (not daily)
Yes, I use smokeless tobacco every day

At what age did you first try smokeless tobacco? _____

Appendix O

Resource Information Handout

If you have questions or would like further information about this study, you may contact Dr. Rebecca Gilbertson at 218-726-7638 or gilbertr@d.umn.edu. Thank you for your participation in this study, and please do not hesitate to contact our research team if you have any questions about this study.

If you have concerns about your psychological well-being or substance use, we encourage you to contact any of the following resources:

- National Suicide Prevention Hotline: 1-800-273-8255
- National Domestic Violence Hotline: 1-800-799-SAFE (7233)
- Substance Abuse and Mental Health Services Administration Helpline: 1-800-662-HELP (4357)
- UMD student counseling services at 218-726-7913 (https://health-services.d.umn.edu/counseling-services)
- Birch Tree Center in Duluth at 218-623-1800 for mental health emergencies (http://www.birchtreeduluth.com/)
- Your personal or family health care provider