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Project Title: MindREaL: Mindfulness for Resilience in Early Life (MindREaL)

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Principal Investigator:
Namik Kirlic PhD, Post-Doctoral Associate, Laureate Institute for Brain Research

Sub-Investigators:
Martin Paulus, MD, President and Scientific Director, Laureate Institute for Brain Research
Robin Aupperle, PhD, Principal Investigator, Laureate Institute for Brain Research
Amanda Morris, PhD

Sponsor: Oklahoma State University and Laureate Institute for Brain Research

ABSTRACT

Early life stress (ELS) is associated with a number of psychiatric and medical conditions later in life, thought to be caused by subsequent disruptions in biological processes involved in regulation of stress responses. Given that these alterations have long-lasting effects, there is a great need for effective preventative interventions. The long-term goal of this project is to identify early interventions that may most powerfully mitigate risk for psychiatric illness among adolescents with exposure to early life stress (ELS), with a focus on interventions that can be widely and effectively implemented, have the potential for long-lasting benefits, and can effectively engage targeted neurobiological processes and networks. The specific aims of the present study are to 1) examine how ELS impacts biological processes associated with regulation of stress, and 2) identify how a mindfulness-based intervention (MBI) impacts affective symptoms and biological processes dysregulated by ELS.

The proposed study will utilize a multi-method design to examine the effect of mindfulness on biological processes (i.e., stress responses) disrupted by exposure to ELS among adolescents age 13 to 15. Adolescents will first complete self-report measures of childhood adverse experiences, trauma, and neglect. Forty eligible adolescents will be next randomly assigned to either an eight-session mindfulness-based stress reduction (MBSR) intervention or treatment as usual (TAU). Pre- and post-intervention assessment will include (a) self-report measures of symptoms and emotion regulation, (b) a blood draw for assessment of inflammatory markers and gene expression, and (c) a stress task with saliva cortisol collected before and after this task.

SPECIFIC AIMS

The current study will accomplish the following goals.

Aim 1: Examine how ELS impacts biological processes associated with regulation of stress. Hypothesis 1a: ELS severity will relate negatively to cortisol changes with the TSST-C. Hypothesis 1b: ELS severity will relate to increased demethylation of the FKBP5 gene and inflammatory markers (IL-6, CRP).

Aim 2: Identify how MBI impacts affective symptoms and biological processes dysregulated by ELS. Hypothesis 2a: MBI adolescents will evidence a clinically significant reduction in internalizing and externalizing symptoms, and emotion dysregulation. Hypothesis 2b: MBI adolescents will evidence normalization in cortisol reactivity in pre- to post-TSST. Hypothesis 3c: MBI adolescents will evidence a normalization in the methylation of the FKBP5 gene and levels of inflammatory markers (IL-6 and CRP).

BACKGROUND AND SIGNIFICANCE

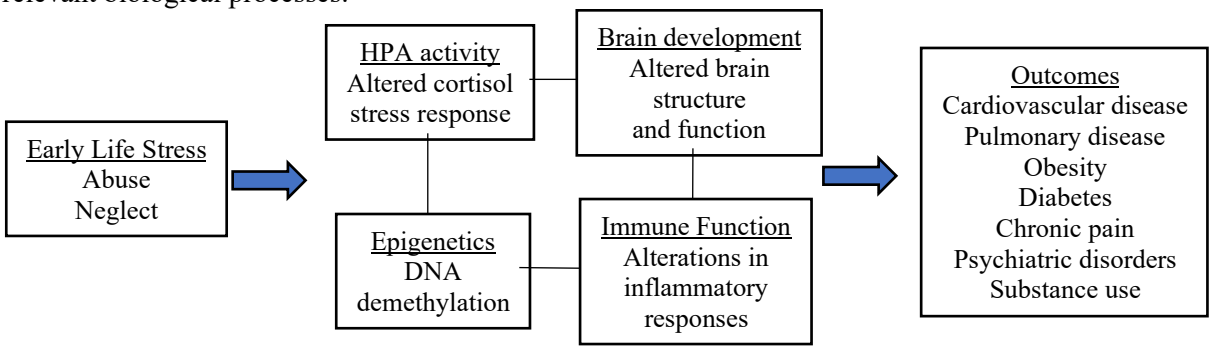
Early life stress, characterized by childhood sexual abuse, and physical and emotional abuse and neglect is the leading preventable risk factor for future adverse physical and mental health outcomes. ELS affects more than 700,000 children and adolescents in the United States [1] and is a serious problem in Oklahoma, where recent reports suggest that 15,187 of youth (~1.5%) are being abused and neglected [2-4]. It is estimated that yearly cases of abuse and neglect may impose a cumulative cost to society of $80.3 billion in direct (e.g., hospitalization, mental health care costs, and child welfare costs) and indirect costs (e.g., special education, early intervention, and lost productivity) [4].

Consequences of ELS exposure are staggering. Several hallmark studies, including the Adverse Childhood Experiences (ACE) study, found that adults with >4 ACEs were at a significantly greater risk for obesity, cardiovascular, gastrointestinal, and respiratory disease, as well as depression, anxiety, panic, suicide attempts, and substance use [5, 6]. Further, ELS is associated with greater severity and comorbidity, earlier onset, and poorer response to treatment of psychiatric conditions [6].

These negative outcomes are believed to stem from a cascade of ELS-related alterations in biological processes responsible for regulation of stress, including epigenetic, HPA axis, brain development, and immune system disruptions [7]. Stress activates the HPA axis resulting in the release of cortisol, necessary for integration of appropriate responses to stress and the subsequent return to homeostasis via a cortisol-

based negative feedback loop. The FKBP5 gene regulates glucocorticoid receptor sensitivity and is thus crucial in this negative feedback loop. ELS exposure and the excessive cortisol release due to the repeated activation of the HPA axis results in the demethylation of the FKBP5 gene, in turn increasing the resistance of the glucocorticoid receptors. This, in turn, diminishes the negative feedback loop, and prolongs stress hormone activation following a stressor. Overtime, this can lead to dysregulated HPA axis functioning (including attenuation of stress responses altogether [8]) and alterations in brain structure and function (e.g., hippocampus) and the immune system. ELS is associated with blunted cortisol responses during a stress induction task [9, 10], as well as disrupted immune system functioning, characterized by increased levels in pro-inflammatory markers [11, 12]. Neuroimaging evidence suggests that ELS alters trajectories of brain development in turn impacting network architecture and circuits involved in threat detection, emotional regulation, and reward anticipation [13, 14]. For example, a recent study showed aberrant amygdala responses to emotional conflict among maltreated youth, and subsequent failure to engage pregenual anterior cingulate (ACC)-amygdala inhibitory circuitry during its implicit regulation [7]. Together, dysregulation of these systems increases the risk for psychiatric, immune, and metabolic disorders [15] (Figure 1).

Figure 1. A representation of how early life stress impacts mental and physical health by affecting relevant biological processes.



Given that these alterations have long-lasting effects, there is a great need for effective interventions. Past intervention studies indicate that ELS adolescents and adults with psychiatric illnesses (e.g., depression) have significantly poorer relative responses to psycho- and pharmacotherapy [16, 17]. However, ELS exposed individuals are underrepresented in intervention studies, and longitudinal treatment outcome studies using multiple objective measurements of change, including biomarkers, are needed.

Regulation of stress and emotions are crucial developmental processes often disrupted by ELS exposure [18, 19]. Given the increased neural plasticity and ongoing brain development, adolescents exposed to ELS may be malleable to long-term normalization in systems subserving stress responses as a result of psychological intervention. Previous research has document such changes. For example, rodent studies show that optimal maternal rearing results in changes in gene expression in offspring resulting in improved stress resilience [20], while neuroimaging studies in adult humans show normalization in activity in the prefrontal cortex and limbic regions involved in stress responses following psychological intervention [21].

Mindfulness-based interventions (MBI) promote emotional awareness and regulation by developing one's ability to observe internal experiences and gain control over thoughts, emotions, and behaviors [22]. Thus, they are well suited to target biological processes involved in stress regulation. Research in both adults and youth shows that MBIs reduce symptoms of depression and anxiety, and improve cognitive and relational outcomes [23-26]. Moreover, recent data indicate that mindfulness practice may

positively influence processes involved in regulation of stress responses in adults, including gene expression (e.g., decreased expression of pro-inflammatory genes) [27], immune and endocrine system markers (e.g., reduction in C-reactive proteins and increased cortisol reactivity, respectively) [22, 28], and activation in a distributed network of brain regions [29-31]. Scant neuroimaging data suggests that mindfulness practice may activate a distributed network of brain regions, including the anterior insula, subgenual and rostral ACC, dorsal and ventral PFC [15, 23, 24], and increase amygdala-ventral PFC connectivity during emotional tasks. Furthermore, the extent of these neural effects relate to the amount of symptom reduction observed[25]. Consequently, it has been proposed that mindfulness exerts its observed psychological and behavioral changes by increasing the activity and functional connectivity of prefrontal cortical regions involved in top-down regulation, while simultaneously decreasing the activity and functional connectivity in neural regions that gate emotional response [11]. Therefore, mindfulness represents a potential regulatory intervention that may inhibit or reverse some of the deleterious long-term effects of ELS exposure.

In summary, previous research suggests the ELS has long-lasting adverse mental and physical health consequences, which likely stem from related disruptions in epigenetic, hormonal, inflammatory, and neuronal systems. At this time, it is unclear whether these disruptions can be compensated for or altogether reversed with psychological interventions. Given the high prevalence of ELS and difficult socioeconomic circumstances in Oklahoma, prevention of ELS adverse outcomes is perhaps one of the most important challenges currently facing our community.

CRITERIA FOR SUBJECT SELECTION

Recruitment

Adolescents participants will be recruited from the Tulsa metropolitan area through Facebook ads, radio ads, and flyers, and contact with local mental health agencies, including Family and Children's Services (FCS) of Tulsa. FCS provides behavioral health care and family services for a range of issues, including trauma exposure and neglect. Currently, they serve more than 400 adolescents ages 13 through 15. Additionally, as part of a consortium, LIBR is currently conducting a large longitudinal study examining adolescent brain and development (ABCD; Western IRB: U01DA041089). Recruitment for the current study will focus on siblings of the ABCD participants of which more than 100 are estimated to be in the 13-15 age range. Finally, LIBR is conducting a neuroimaging study of adolescents and their parents (DIBS; Oklahoma State University IRB: 2017011). Participants excluded from this study (e.g., due to orthodontic braces) may be eligible for the current study. If these participants consent to being contacted by other studies conducted at LIBR, they will be recruited. Recruitment materials will be distributed through mailings, placement in treatment/research facilities, and contact with treatment providers. Recruitment materials are included with this application.

The racial and ethnic demography in the present study is aimed to match the demographic composition of Tulsa, OK based on the U.S. Census Bureau, 2010 Census [32]. The expected racial composition is: 69% White and 11% Latino, 10% Black/African-American, 2% Asian, 6% American Indian/Alaskan Native, and less than 1% Pacific Islander.

We will be recruiting up to 80 participants for this study. The advertisement materials will indicate that LIBR is conducting a research study designed to better understand how mindfulness training may help teens deal with stress and become more resilient. The materials will explain that the mindfulness training will consist of 8 biweekly sessions that have been known to help teens manage stress. Additionally, recruitment materials will state that a number of participants will be invited to provide biological samples (i.e., saliva and blood) to examine their relationship with early positive and negative life events and impact of mindfulness on biological responses. We aim to recruit 40 participants with ELS for the research study.

Inclusion Criteria

- Age 13.00 to 15.99 years at time of baseline assessment
- Able to validly and safely complete baseline assessments
- All genders
- All races
- Eligibility as a subject with early life stress will be determined by:
    - Scoring 4 or greater on Adverse Childhood Experiences (ACE) scale, with at least two experiences having occurred prior to age 10.

Exclusion Criteria

- No biological parent or legal guardian identified to give permission for minor to participate
- History of neurological disorders including seizure disorder, cerebral palsy, or other conditions requiring neurological or medical care, being managed for migraines (e.g., daily prophylactic medication, seeing a neurologist for migraines), or a diagnosis of Developmental Delay, including severe learning disorder, mental retardation, pervasive developmental disorder, or other conditions requiring repeated and persistent specialized education.
- Current psychotic disorder, bipolar disorder, obsessive-compulsive and related disorders, substance use disorder, or conduct disorder.
- Current active suicidal ideation.
- Current use of medications with major effects on brain function or blood flow (e.g., antipsychotics, mood stabilizers); ADHD medications and SSRIs, that have been stable for at least 6 weeks, are not exclusionary since their use is associated with conditions that confer risk for monitored disorders that emerge in adolescence, and assessment of these individuals will provide useful data to the scientific community. Youth on ADHD medications and SSRIs will not be asked to go off their medications.
- Not fluent in English
- Non-correctable vision, hearing or sensorimotor impairments, as protocol elements may not be valid.
- Youth planning to move to an area not within reasonable traveling distance of LIBR; knowledge at baseline that treatment completion / follow-up will not be possible.
- Youth appears to be high/intoxicated, or withdrawing from the effects of alcohol or drugs at time of enrollment.
- Youth / parent who are unable or unwilling to provide biological samples (i.e., blood draws or saliva collection).
- Female youth who are pregnant
- Youth who are currently in unsafe environments (e.g., currently living with an abusive parent)

Eligibility will be determined by the PI and trained study staff during screening and baseline assessments.

Informed Consent

Interested youth or parents will call the research site in response to recruitment advertisements or the parent will be contacted by study staff using contact information provided by each agency/study. Verbal consent and permission will be obtained from a parent/legal guardian initially, and the mindfulness training and the research study will be described in more detail. The parent/legal guardian will be screened with basic eligibility questions using the Parent Screening Questionnaire. If the family remains interested and the youth remains eligible, an in-person assessment session will be scheduled during which

documented HIPAA, Parent/Legal Guardian, Permission for Child, Adult (parent, legal guardian, primary caregiver) Informed Consent, and Youth Assent forms will be reviewed with the family.

All participant interactions including consenting will be conducted in private interview/exam rooms at the Laureate Institute for Brain Research. These exam rooms are secured from public areas via combination locked doors that are only accessible to authorized personnel. Consent and parent permission from the enrolled parent will be obtained by members of the research team that have received training to obtain consent for this study. Written informed consent will be obtained from each participant after they have been provided a full verbal and written explanation of the study purposes, procedures, risks, and benefits, and after they have been allowed sufficient opportunity to review this information and ask questions concerning any aspect of the study. The researcher will remind the subject that participation is strictly voluntary and remind them that they have the right to withdraw at any time without penalty. Family members will be allowed to be present and discuss the consenting process with the participant if requested.

During the informed permission and consent process, the parent/legal guardian/primary caregiver will be told he/she will not be informed about the child's substance or medication use and that youth self-report and lab data are confidential, with the exception of any acute safety issues (e.g., suicidality, medical emergency). Recruitment related research procedures present no more than minimal risk of harm to subjects and involve no procedures for which written consent is normally required outside of the research context. The assent/consent forms include baseline assessments, intervention sessions, and post-intervention follow up participation; therefore, informed assent/consent will be obtained at study entry. However, if the assent/consent forms are amended during the follow up phase of the study, participants will be asked to again provide assent/consent/permission. After providing their assent to participate, we will ensure youth fully understand the study and the assenting process by administering a Consent Quiz consisting of simple questions about the procedures explained in the Youth Assent.

Enrollment

Eighty adolescents will be enrolled. Randomization will be stratified by ELS exposure, gender, and medication use. High-ELS adolescents (n=40) will complete both self-report assessments and biospecimen / behavioral tasks portions at baseline and follow-up visits, while low-ELS adolescents (n=40) will complete only self-report assessments at baseline and follow-up visits. Twenty high-ELS and 20 low-ELS adolescents will then be randomly assigned to an eight-session mindfulness-based stress reduction (MBSR) intervention or treatment as usual (TAU). Adolescents will be informed during the consent that only some participants will be asked to complete the more extensive assessments, but they will not be told how that determination is made (so as to prevent participants from reporting higher ELS in order to obtain more compensation in the study). To remain in the study, participants will be required to complete 6 of 8 mindfulness sessions.

Participation Compensation

Adolescent compensation will be as outlined in the table below. Snacks will be provided per request, depending on the duration of the study appointment. For subjects who do not complete the baseline or follow up visits, they will receive a prorated amount of $10 per hour of participation. Compensation for weekly assessments will be made following completion of each assessment. The payment will be given as a ClinCard, a reloadable credit card that can be used at stores, online, or for cash at local banks.

	Baseline	Online Assessement 1	Online Assessment 2	Sessions 1 - 8	Follow up	**Total**
High ELS (n=40)	$90	$5	$5	$40	$80	**$220**
Low ELS (n=40)	$20	$5	$5	$40	$20	**$90**

RESEARCH DESIGN AND METHODS

Overview

At the baseline visit, adolescents will complete a number of surveys assessing positive and negative life events, childhood trauma history, mental health symptoms, emotion awareness and regulation, resiliency, and mindfulness skills. At this time, adolescents will be randomly assigned to either a MBSR training group or online assessment only (TAU) group. Particular care will be taken to ensure that 20 adolescents with ELS are assigned to each group. ELS adolescents in both groups will also complete behavioral tasks and provide biospecimens.

MBSR training will commence within two weeks of baseline assessment and consist of 8 sessions over 4 weeks. Each week, training participants will complete a brief self-report assessment electronically. TAU group will complete similar assessments online.

Following mindfulness training sessions, a follow-up visit will be scheduled during which baseline procedures will be repeated. Adolescents and parents may be contacted via text message to remind them of upcoming sessions as well as reminders to complete online portions of questionnaires.

Self-Report Assessments

Parent characteristics will be assessed, including age, educational attainment, race and ethnicity, and marital status. Current family income will be obtained, as well as sources of subsidized income such as WIC, food stamps, AFDC (TANF), Social Security, or Disability. Child age, gender, race and ethnicity will be assessed.

History of ELS will be assessed using the *ACE Study Questionnaire* [33]. This measure assesses which assesses instances (answered yes or no) of physical abuse, sexual abuse, emotional abuse, neglect, and household dysfunction before the age 18. Household dysfunction consists of living with a substance abuser or a parent with mental illness, witnessing domestic violence, having parents divorced or separated, or having a family member go to prison.

Protective and Compensatory Experiences (PACEs) assess ten experiences children need to prevent adverse childhood experience and promote resilience, including stable caregiving, stable home and school environments, positive adult and peer relationships, and involvement in extracurricular activities.

The Life Chart (LC) is a structured interview that is used to obtain life history related to significant negative or positive events and the associated mood ratings in a person's life.

Traumatization history, including its severity, will be assessed with the *Childhood Trauma Questionnaire (CTQ; [34])*. The CTQ consists of 28 items, comprising five subscales: (1) Physical abuse, (2) Sexual abuse, (3) Emotional abuse, (4) Physical neglect, and (5) Emotional neglect.

Adolescents' depressive symptoms will be assessed with the 13-item *Short Mood and Feelings Questionnaire – Child Version (SMFQ-C; [35])*. A range of other internalizing and externalizing symptoms will be assessed with the widely-used *Youth Self Report (YSR)* scales [36].

Adolescents will also complete the *Sadness and Anger Management Scales (SAMS; [37])* to assess emotion regulation. Behavioral responses for each emotion are rated for dysregulated expression (e.g., "I

cry and carry on when I'm sad"), and coping (e.g., "I try to calmly deal with what is making me feel mad"). Difficulties in *Emotion Regulation Scale (DERS)*, a comprehensive measure of emotion regulation and dysregulation, will assess the following domains: a) awareness and understanding of emotions, b) acceptance of emotion, c) the ability to engage in goal-directed behavior / refrain from impulsive behavior, and d) access to effective emotion regulation strategies [38].

Mindful Attention Awareness Scale – Adolescent (MAAS-A; [39]) is a self-report measure that assesses mindfulness in adolescents across 14 items, such as "It seems I am 'running on automatic' without much awareness of what I'm doing." This is the adapted version of the adult measure, which has been found to be sensitive to change in mindfulness as a result of MBSR training.

Connor-Davidson Resilience Scale (CD-RISC 10; [40]) is a brief measure that assesses stress coping abilities. This measure was found to have adequate reliability and validity. However, subsequent factor analyses demonstrated that a briefer version of the measures, utilizing just 10 items was sufficient to obtain the same information as that obtained from the full scale.

Adolescent Alcohol and Drug Involvement Scale (AADIS; [41]) will assess adolescent history of drug use for a range of alcohol and non-prescription drugs.

Suicide Behavior Questionnaire – Revised (SBQ-R; [42]) has four items that assess for previous suicidal ideation and attempts, frequency of suicidal ideation, threat of a suicide attempt, and likelihood of suicidal behavior in the future. This questionnaire had been used and validated with adolescent and college samples. A cutoff score of 7 has been suggested as associated with risk for suicidal behavior and further follow-up. In order to take a more conservative approach, we will follow-up with any student scoring >7 total score, or >2 on question #4 (indicating they are unlikely to very likely to commit suicide someday). The timeline for the questions will be revised to specify the time duration since their last survey (rather than "in the past year") – for example, "since your last survey 3 months ago". Those who score greater than these specified cutoffs will be contacted by the PI or other clinically-trained staff to further assess for suicidal ideation, plan, and intent using the Columbia-Suicide Severity Rating Scale.

Columbia-Suicide Severity Rating Scale (CSSR; [43]) supports suicide risk assessment through a series of simple, plain-language questions that anyone can ask. The answers help users identify whether someone is at risk for suicide, assess the severity and immediacy of that risk, and gauge the level of support that the person needs. In cases of a positive screen for suicidal ideation (see above description of the SBQ-R), the full CSSR Interview (last month only) will be completed by the PI or other clinically-trained staff to further assess suicidal ideation, plan, and intent.

Adolescents will report on unsupportive parenting using the punitive parenting/harsh discipline scale of the Alabama Parenting Questionnaire (APQ; [44]), which includes questions assessing *corporal punishment and hostility*.

<u>Behavioral Tasks</u>

ELS participants will complete a standardized task that induces stress reactions, as well as an emotional conflict task, at baseline and follow-up time points.

Trier Social Stress Test for Children (TSST-C; [45]) is the adapted version of the TSST for the use in children and adolescents aged 7-16 years of age. Participants will be instructed that this task consists of two portions, a public speaking and mental arithmetic components. The speaking task begins with the adolescent given the stem of a story, and he or she will be asked to complete the story in an interesting and exciting way. Additionally, the adolescent will be told that the story ending should be better than that provided by other participants. The participant will have a 5-minute preparation period and then must complete the story over the course of 5 minutes. If the adolescent finishes the story in less than 5 min, they will be asked to continue, and this request is delivered in a friendly, supportive manner. Following the story component, the adolescent will complete a serial subtraction task, with encouragement to complete it as quickly and as accurately as possible. Salivary cortisol will be collected before and 10-20

minutes after the task. TSST-C will be delivered by two research assistants on the study.

During the *Emotion Conflict Task (ECT*; [46]) participants will view images of happy or fearful facial expression, overlaid with the words "FEAR" or "HAPPY". Participants will be instructed to identify the underlying facial emotional expression (fearful or happy) while ignoring an overlying emotion word ('FEAR' or 'HAPPY'). Trials will vary such that emotional distracter words either match ('congruent' (C)) or conflict ('incongruent' (I)) with the underlying facial expression. The task will be adapted for children by utilizing an established set of child emotion-face stimuli of varied ethnicities, ages 10–17 years [47].

During the *Learning and Decision-making Task*, participants will make trial-by-trial predictions about task outcomes, following which the actual outcome is shown. Participants make choices based on accumulated knowledge after each new trial. The task will examine learning and decision-making processes in a controlled environment.

Presentation of behavioral tasks will be counterbalanced across participants.

Physiological Monitoring

Adolescents will be fitted with an Equivital Life Monitoring Sensory system (an ambulatory, multi-sensor, continuous monitoring vest for collecting, analyzing, and reporting health data.) The Equivital System is able to collect reliable objective physiologic data through various sensors, which measure electrical activity of the myocardium via a 3-lead EKG, and activity/posture via a two-axis accelerometer. The sensor array of the Equivital System is embedded in a strap worn across the chest, made of washable material that fits snugly and can be worn comfortably for extended periods by individuals of varying height and weight.

Biospecimens

Saliva and blood samples will be collected at LIBR and transported for processing and storage to the Biomarker Core at the OU-Tulsa Integrative Immunology Center. Saliva will be collected using passive drool SalivaBio collection tubes (Salimetrics) and then centrifuged, aliquoted and stored at 80^{o}C. Blood will be collected using standard CPT (for PBMC isolation) and serum vacutainer tubes. A total of about 200 mL (less than 14 tablespoons) of blood will be drawn during the entire study by a trained phlebotomist. PBMCs will be aliquoted and stored in liquid nitrogen and serum will be aliquoted and stored at 80^{o}C. Biomarker assays will be performed at the CIRCA Biomarker Core. Cortisol concentrations in saliva samples will be determined using Salimetrics High Sensitivity Salivary Cortisol EIA kits. IL-6 and CRP levels in the serum samples will be assayed using MesoScale Discovery V-PLEX assay kits. RNA will be isolated from PBMCs and a cDNA bank will be created. Gene expression analyses by qPCR for a project specific gene and one control gene for each subject at baseline and follow up will be done.

TREATMENT

Mindfulness-based Stress Reduction for teens (MBSR-T; [48, 49]) is an adapted version of adult MBSR. It consists of 8 weekly 1.5 hour sessions. Sessions are focused around three core elements: (1) intention (direction of effort toward mindfulness practice), (2) attention (experiencing what is taking place in the moment rather than categorizing or ruminating upon events based on memory), and (3) attitude (nonjudgmental or open experience of even difficult cognitive, emotional, or somatic experiences). Adolescents will receive a workbook to reinforce instruction, and a CD with sitting and body scan mediations for at-home practice (consisting of 20-35 minutes daily). TAU will consist of any treatments or activities in which the enrolled participant is engaged, including psychotherapy, pharmacotherapy, or other services provided through various agencies. TAU participants not connected with services will be given appropriate referrals. MBSR-T participants will not be asked to discontinue any other intervention for the duration of the study.

MBSR-A will be delivered by the PI (Namik Kirlic, PhD; a clinical psychologist expected to be fully

licensed in the state of Oklahoma by the start of the study) and a clinical psychologist in training (a practicum student in the University of Tulsa clinical psychology doctoral program), supervised by a licensed clinical psychologist (Dr. Robin Aupperle, project mentor). Dr. Kirlic will receive training in MBSR-T delivery via the online training conducted by the MBSR-T creator, Gina Biegel, LMFT (https://www.stressedteens.com). Clinical psychologists in training will be provided training and supervision in the treatment protocols from Dr. Kirlic. A treatment manual will be developed for the purposes of training and adherence.

Measures will be taken to promote treatment fidelity, according to previous recommendations [50]. Each session will be recorded and up to 20% sessions will be randomly selected for fidelity ratings by research staff and expert consultants. Skill acquisition and fidelity will be assessed using CBT competencies developed for the Improving Access to Psychological Therapies (IAPT) Program [51]. Subject compliance will be assessed using (1) session attendance, (2) Homework Rating Scale to assess between-session compliance and (3) Working Alliance Inventory for Children and Adolescents (WAI-CA) therapist and client versions, to assess subject compliance and therapist behavior intra-session [52]. SMFQ-C will be completed weekly to assess symptom severity.

GENDER/MINORITY/PEDIATRIC INCLUSION FOR RESEARCH

The study will include 80 adolescents ages 13, 14, and 15. This is justified by the focus of the study on the biological processes associated with ELS exposure in adolescents and their changes as a result of treatment, and will allow for meaningful analyses. The selection of this age range ensures that the adolescents have entered into puberty, thus eliminating potential confounds of pubertal development on the data. Women and minorities will be included in the study without prejudice according to their representation in the study population. Subjects will be recruited from the greater Metro Tulsa area and should thus share the racial and ethnic composition of this area. All efforts will be made to ensure that our subject population closely resembles the gender, ethnic, and racial composition of the greater Tulsa area.

PARTICIPANT RISKS AND PLANS TO MINIMIZE RISKS

Risks associated with coercion

Risk: Undue pressure on youth to provide informed assent

Risk minimization: Youth assent will be obtained separately and independently from parental consent, and as both are required for the youth to participate, the risk of coercion because of the youths' minor status is limited.

Risks associated with Screening and Evaluation

Risk: Some of the questions in the interviews may be distressing or uncomfortable to answer due to their personal or emotionally relevant nature.

Risk minimization: The researchers are trained to frequently inquire the subjects about their willingness and ability to continue with testing. If the participants express concerns about continuing with testing, the research assistants will stop testing, offer a break, or, in case the subject is not willing to continue, terminate the testing session. If subjects report psychological distress, suicidal ideation, or intent to harm self or others, Dr. Kirlic or Dr. Aupperle, will be contacted immediately to ensure appropriate care and compliance with mandated reporting to authorities. Subjects may be referred for professional intervention, as deemed appropriate, including calling emergency personnel (911) if needed. A current list of local mental health treatment programs will be provided to all subjects at screening. Information reported will be kept in confidence with the exception that disclosure of suicidality, homicidality, or child or elder abuse warrant reporting to appropriate authorities.

Risks associated with blood draw and saliva collection

Risk: Blood draws may be associated with mild pain, bruising, and infection at the puncture site as well as possible risk of fainting or dizziness.

Risk minimization: Adolescents will be told about the potential discomforts associated with blood draws and saliva collection. Samples will be taken by a trained phlebotomist and the subject will be informed that the blood draw may be associated with mild pain, bruising and infection at the puncture site, as well as a possible risk of fainting or dizziness.

Adolescents and parents/guardians will be informed that this research study will not include diagnostic testing of genetic information to confirm any findings that may result from self-report findings. Any incidental findings of clinical significance will be shared with the parent/guardian to follow up with a physician. There is a minimal risk of undue stress or concern if the finding is determined to be benign or not clinically significant. Furthermore, data from this study, including genomic information likely to be generated in future analyses, will be available in controlled-access data repositories for broad sharing. While these repositories will not contain personally identifying information, it is possible that the participants' genetic or medical information in these databases could be linked to them. For example, someone could compare information in the databases with information from relatives residing in another database and be able to identify the participants. Some genetic variations can help predict future health problems or can be used by law enforcement agencies to identify a person or his/her blood relatives. Therefore, participants' genetic information potentially could be used in ways that could cause him/her or the family distress, such as by revealing that a child (or a blood relative) carries a genetic disease. There also may be other privacy risks that we have not foreseen. A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against individuals based on their genetic information. This law generally will protect the participants in the following ways:

- Health insurance companies and group health plans may not request genetic information obtained in this research.
- Health insurance companies and group health plans may not use genetic information when making decisions regarding eligibility or premiums.
- This new Federal law does not protect against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Risks associated with behavioral testing

Risk: The subjects may experience feelings of discomfort and fatigue due to mild to moderate demands on the attention and cognition, as wells as feeling uncomfortable or anxious due to the stressful and emotional nature of the stimuli used during the tasks, including the social stress task.

Risk minimization: The researchers are trained to frequently inquire with the participants about their willingness and ability to continue with testing. If the participants express concerns about continuing with testing, the research assistants will stop testing, offer a break, or, in case the participant is not willing to continue, terminate the testing session. If participants report psychological distress, suicidal ideation, or intent to harm self or others, Dr. Kirlic or Dr. Aupperle will be contacted immediately to ensure appropriate care and compliance with mandated reporting to authorities. Participants may be referred for professional intervention, as deemed appropriate, including calling emergency personnel (911) if needed. A current list of local mental health treatment programs will be provided to all subjects at screening. Information reported will be kept in confidence with the exception that disclosure of suicidality, homicidality, or child or elder abuse warrant reporting to appropriate authorities.

Risks associated with treatment

Risk: it is not expected to occur, but participants may experience adverse effects to treatment in the form of worsening symptoms.

Risk minimization: First, adolescents will be informed that there are no known risks associated with participating in mindfulness-based psychological interventions. We will share with them that these interventions have been shown to have a positive impact on psychological well-being, including a reduction of psychological symptoms and emotional reactivity, and improved emotion regulation. Symptoms will be monitored weekly, and in case of worsening, we will follow the contingency plan as outlined below.

For adolescents who wish to or are in need of treatment for psychological symptoms, trauma, or life stress, an alternative to participation in this study would be to participate in a different type of treatment available through the community. Mindfulness interventions have been shown to reduce symptoms of psychopathology in a range of samples, and therefore many of the adolescents are expected to benefit from it. TAU condition is necessary to delineate the effect of mindfulness intervention on biological processes related to ELS. In both conditions, adolescents will not be asked to stop any intervention that they are currently undergoing to participate in this study. An alternative to participation for healthy controls would be to not participate; however, they will also be provided with mental health and substance abuse resources in the community. Finally, all adolescents will be provided with referral information.

If an adolescent should become uncomfortable during any procedures, he/she can stop the procedures or withdraw from the study at any time. If any medical problem arises, 911 will be contacted immediately. If psychological distress, suicidal ideation, or another problem should occur, the designated licensed professional (MD or Ph.D.-level clinical psychiatrist/psychologist) will be contacted immediately. Adolescents with significant substance use or psychopathology (i.e., psychosis, bipolar disorder) are excluded at baseline; however, substance use and psychopathology may emerge during the course of the study. In such a case, we will follow the contingency plan as outlined below.

Contingency plans for monitoring suicidality, worsening clinical symptoms, or other mental health emergencies

All participants who are deemed a serious suicide risk will be excluded from the study. In the evaluation of suicidal risk, we will exclude from participation any adolescent who endorses having developed a plan or intent to attempt suicide, or has made a serious suicide attempt within the preceding six months. Any volunteer who is excluded from participation for these reasons will be referred for emergency care according to written LIBR policies for managing potentially suicidal patients, as described below.

While the participants are in this study they will be monitored for the development of suicide risk or for worsening in their clinical illness by a clinician. This will be conducted using the weekly self-report assessments and clinical observation, as described above. If concerns arise when the participant is physically present at the Laureate Institute for Brain Research, psychologists Namik Kirlic, PhD and Robin Aupperle, PhD are available on site to address any concerns that arise. If Dr. Kirlic or Dr. Aupperle are unavailable or further evaluation is deemed necessary, psychiatrists Drs. Martin Paulus, and Sahib Khalsa will be available.

For participants who are at the LIBR facility and are deemed to constitute a serious risk for suicide, the LIBR policy requires that they be escorted by two clinicians to the onsite, 24-hour emergency facility at Laureate Psychiatric Clinic and Hospital (which is located about 100 yards from the LIBR facility). If participants refuse to be escorted to this facility and leave the LIBR premises, the study clinician will

contact the Community Outreach Psychiatric Emergency Services (COPES – 918 744 4800), which is available 24 hours per day to send a mobile unit to the person's home.

For participants who develop serious suicidal ideation while not on the LIBR premises, these participants will be instructed to call 911 or to go to the nearest emergency room if they feel they are a threat to themselves or others. They will also be given the contact details of the Tulsa Community Outreach Psychiatric Emergency Services (COPES – 918 744 4800).

If adolescent presents intoxicated during either the research or treatment component of the study, he or she will not participate in that visit. The parent will not be informed of the positive results, unless the intoxication is life-threatening. To ensure the youth's safety, he/she will be asked to remain on the premises until his/her breathalyzer reading is 0.00/ saliva re-test is negative. If a safe drive with their parent/legal guardian is not available, transportation (i.e., taxi service) will be arranged from the research site back to the youth's address. If results are not those anticipated by the adolescent (i.e., causing distress), PI will discuss the test results with the participant with the objective of reducing their concerns.

For participants who have clinical issues that do not reflect a psychiatric emergency, they will be given a telephone number where they can reach a LIBR clinician during those hours when LIBR is officially staffed by clinicians (weekdays between 0800 and 1700). For weekends and off-hours (evenings and nights) they are provided a second telephone number where they can reach the 24-hour per day on call service for LIBR, which is provided through the Call Center of Laureate Psychiatric Clinic and Hospital.

Upon study completion, participants who are currently under treatment by an external provider will be referred to their own clinician. Participants who are dropped from study participation, or participants who complete the study and are not under the care of a clinician, will be provided a referral to a psychiatrist or other mental health professional at one of the following clinics (regardless of the reported level of post-treatment symptoms):

Outpatient (insured)
Laureate Psychiatric Clinic and Hospital
6655 South Yale Ave
Tulsa OK, 74136
(918) 481-4000

Department of Psychiatry (sliding scale)
University of Oklahoma College of Medicine
Tulsa OK, 74136
(918) 619 4400

Outpatient (uninsured)
Tulsa Center for Behavioral Health (sliding scale)
2323 South Harvard Ave
Tulsa, OK, 74114
(918) 239 2100

Associated Centers for Therapy (sliding scale)
7010 South Yale Ave, Suite 215
Tulsa OK, 74136
(918) 492 2554

Inpatient
Laureate Psychiatric Clinic and Hospital

6655 South Yale Ave
Tulsa OK, 74136
(918) 481 4000

Shadow Mountain Behavioral Health System
6262 South Sheridan Road
Tulsa OK, 74133

Individually tailored referrals will be made for participants who reside outside of the Tulsa region, so that they will be referred to psychiatric services that are located near their home or workplace.

Potential Benefits

Participation in this study involves minimal risk for adolescents, yet has the potential for providing substantial long-term benefits to them, as well as toward understanding the extent to which symptoms, emotion regulation, epigenetic factors, and functioning of the HPA axis associated with ELS are mitigated by mindfulness-based intervention.

Risk/Benefit Ratio

Given the minimal risk to adolescents involved, the prevalence of ELS exposure and its substantial short- and long-term adverse psychological and health outcomes, positive psychological impact of mindfulness intervention, and the information gained from this study on how a range of psychological and biological processes dysregulated by ELS may change as a result of intervention, we believe that the risk/benefit ratio is acceptable.

Expense to Participants

The only anticipated expense to adolescents and their families is the amount of time spent in research / intervention.

DATA AND SAFETY MONITORING PLAN

Data safety and monitoring will be carried out to ensure and maintain the scientific integrity of this project and to protect the safety of adolescents. Data and Safety Monitoring Board will consist of the PI, primary (Dr. Robin Aupperle) and co-mentors (Dr. Martin Paulus and Dr. Amanda Morris), and research assistant. Review of accumulated outcome data for groups of adolescents will be carried out to determine if any of the procedures practiced should be altered or stopped. Study will be stopped in case of a a significant adverse event or majority of participant showing a worserning of symptoms. Continuous, close monitoring of participant safety will prompt reporting of safety data (i.e., adverse/serious adverse events) to the local IRB. Serious adverse events will be reported to the IRB within 48 hours of the time project staff become aware of the incident. The PIs will provide a summary of the safety conduct of the study to CIRCA as part of the progress report, which will parallel the written report required by the IRB as part of the annual IRB renewal. The review of data and procedures may result in early termination of the study, amendment to the protocol, or changes to the data collection plan. Should the protocol or data collection plans be amended as a result of data review, the relevant IRBs will be notified and the amendment approved prior to study amendment implementation. In addition, the participants will be notified of any significant new findings that develop during the course of research (e.g., other potential risks) that may affect their wish to continue participation in the study.

Each subject will be given a unique identifier with a code. Information for each participant is entered into the Laureate Institute for Brain Research subject database and they are automatically given a LIBR ID e.g. AA001. The code key that links the unique identifier to the subjects' names is kept in a separate file.

All data analysis is performed on de-identified data. Other than the PI, there is no need for personally identifying information to be known to other investigators.

All research documents containing Personally Identifiable Information (PII) and collected research data will be stored electronically on the LIBR Network and/or REDCap. Access to the LIBR Network and to REDCap is granted only to authorized personnel. The LIBR Network is protected by the Palo Alto PA-5250 Layer 7 firewall with licenses for Wildfire, AV, Threat Prevention, and URL filtering. All LIBR Network data is stored on site. REDCap is a secure web application for building and managing online surveys and databases. While REDCap can be used to collect virtually any type of data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments), it is specifically geared to support online or offline data capture for research studies and operations. The REDCap Consortium, a vast support network of collaborators, is composed of thousands of active institutional partners in over one hundred countries who utilize and support REDCap in various ways.

Study information will be made available to the subjects when there has been sufficient data analysis to make reasonable aggregate conclusions. We can share general study characteristics (how many people were recruited, age, primary disorder etc.) early in the process, but will not share study results with subjects until the quality of the data is close to publication level.

If the PI leaves LIBR, and agreement will be made between LIBR and the PI new institution to transfer the data so that the study can continue.

CONFIDENTIALITY

Confidentiality risks are important are to consider in this study. This study collects a variety of sensitive data, in particular with respect to the risk of disclosure of childhood abuse and neglect, illicit substance use, and genetic, mental health, and physical health information. Since personal information is gathered, there exists risk of possible invasion of privacy. Procedures described below minimize the possible breach of confidentiality and invasion of privacy.

Participants will be assured that records and samples will be kept confidential in research files located in a locked office and/or entered into a password-protected computer located behind a secure and maintained firewall. All forms of data described above will be recorded on computerized or paper and pencil data forms that do not contain identifying information. All biological samples (including saliva for genetic and hormone analysis) will be de-identified, and those collected for future analysis will be securely stored on site in locked cabinet and/or freezer, or shipped to the designated lab. Breach of confidentiality is highly unlikely as all personally identifying information will be kept separate from other data collected, and will be linked only by a master subject identification list which is maintained by the project coordinator and PI. Confidentiality may be broken in case when disclosure is required by the law, including disclosure of suicidality, homicidality, or child or elder abuse which warrant reporting to appropriate authorities.

All participants will be informed about the limits of confidentiality, including that the information provided for this research study will not be shared a) between youth and parent (legal guardian, caregiver) with the exception of situations concerning safety (e.g., suicidal during a study session) or b) with the youth's school. No personally identifying information will be coded on the questionnaires, interviews, biological samples, brain imaging data, or scoring sheets. Subject identification numbers are assigned to each participant. Only the PI and limited research staff have access to the file that links subject name with subject number. All data are stored in locked file cabinets in locked offices or password-protected computers located behind secure and maintained firewalls.

Breach of confidentiality is highly unlikely because all personally identifying information will be kept separate from data collected, and will be linked only by a master subject identification list maintained by the lead research associate and the PI. We will inform both youth and parents from the start of the participation and at each follow-up that we will not inform the parents of what the youth informs us

concerning substance use. We will provide all participants and parents with information about substance abuse and mental health services in their area, including free and youth specific services. Parents do have the right to request copies of some types of protected health information. However, as suggested in the *NIAAA Alcohol Screening and Brief Intervention for Youth: A Practitioner's Guide*, we will make it clear, prior to any such information release, that reading this information may compromise our ability to work with the youth and make it more difficult to elicit honest and accurate information. We will inform parents, and other authorities as required by the situation, if we learn that a minor is a danger to self or others.

All research staff will complete on-line training in human subject research, HIPAA, FERPA, and clinical practices, training in respective labs on research data management and confidentiality, and training to criterion on project protocol. As an additional protection to participants, we will seek a a certificate of confidentiality from the NIH for this study.

ANALYSES AND POWER.

Statistical analysis will be carried out using R statistical package. Robust methods, non-parametric tests, or data transformations will be used to address any issues of non-normality. With N=20 per group and 20% attrition, we will have 80% power to detect medium size effects (*f*=.25) between groups from baseline to follow up (Aim 2), and pre- to post-TSST cortisol differences (Aim 2). We are 80% powered to detect large effect sizes (*r*=.5) for regression analyses (Aims 1 & 2). While potentially under-powered for regression and more complex analyses involving additional covariates, results will be crucial for estimating effect sizes for future study design and grant applications.

Aim 1: Examine how ELS impacts biological processes associated with regulation of stress. *Hypothesis 1a: ELS severity will relate negatively to cortisol changes with the TSST*. *Hypothesis 1b: ELS severity will relate to increased demethylation of the FKBP5 gene and inflammatory markers (IL-6, CRP).* Paired-samples t-tests will evaluate the differences in levels of saliva cortisol pre- to post-TSST (cortisol reactivity). Huber robust regression analyses will examine the relationship between intensity of ELS exposure and cortisol reactivity and inflammatory markers.

Aim 2: Identify how MBI impacts affective symptoms and biological processes dysregulated by ELS. *Hypothesis 2a: MBI adolescents will evidence a clinically significant reduction in internalizing and externalizing symptoms, and emotion dysregulation. Hypothesis 2b: MBI adolescents will evidence normalization in cortisol reactivity in pre- to post-TSST. Hypothesis 3c: MBI adolescents will evidence a normalization in the methylation of the FKBP5 gene and levels of inflammatory markers (IL-6 and CRP).* We will use linear mixed effect (LME) analyses (with subject and time entered as random effects) to examine changes from baseline to follow-up time points between the MBSR and TAU groups.

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