

Racial Discrimination and Stress Response

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Study Protocol

1. Significance

U.S. Census Bureau (2018) predicted the United States to become a minority majority nation with the minority population outnumbering the White population in 2044 and minority youth outnumbering White youth in the year 2020. Nevertheless, as compared to White peers, ethnic minority youth face a myriad of social challenges due to their minority status, racial discrimination being a major one. Racial Discrimination, defined as unfair treatment attributed to one's race or ethnicity, is reported to be common among ethnic minority youth in schools and in many other public spaces (Kessler et al., 1999; Rosenbloom & Way, 2004). Racial discrimination is linked to ethnic minority youth's physical and mental health problems, such as depression, anxiety, low self-esteem, behavioral problems, and general well-being (see Priest et al., 2013 for a review), and can have life-long adverse impact into adulthood (e.g., Adam et al., 2015; Assari et al., 2017; Njoroge et al., 2021). Early life racial discrimination can compose significant risks to set ethnic minority youth on a life trajectory of impaired health.

Several explanations have been postulated to explain the deleterious impact of racial discrimination on ethnic minority youth's health (e.g., Sanders-Phillips, 2009). A prominent one in health psychology conceptualizes adverse environmental factors, including racial discrimination, as stressors, the experience of which can elicit physiological arousal and heightened stress response (e.g., McEwen, 2012; Brondolo et al., 2008). Overtime, chronic stress will result in altered dysregulated stress response and make individual susceptible to health problems (e.g., McEwen, 1998). Thanks to the technological advancement, biological response to stressor has been increasingly assessed and recognized as the pathway to predict health outcomes, such as hypertension (e.g., Panaite et al., 2015), diabetes (e.g., Black, 2003) and mental health disorders (e.g., Chojnowska et al., 2021; Walker et al., 2017).

Evidence regarding the role of racial discrimination and dysregulated stress response in ethnic minority population is emerging (see Berger & Sarynai, 2015; Busse et al., 2017; for reviews). For example, Saban and her colleagues (2018) employed a widely used stress paradigm, Trier Social Stress Test (TSST; Kirschbaum et al., 1993), where African American and White American women participants experienced social evaluative stress by preparing and delivering a public speech. The study demonstrated that women who reported higher levels of perceived discrimination had higher levels of inflammatory responses to TSST. However, like many other studies, it only assessed stress response to social evaluative stress rather than racial stressor. Racial stressors, as compared to nonracial stressors, are more prevalent to ethnic minority population and can elicit heightened stress responses in them (e.g., Guyll et al., 2001; Lepore et al., 2006). Biological responses to racial stressors can provide more direct and informational window to reveal the implications of racial discrimination in ethnic minority population's stress regulation and health. Furthermore, most existing studies examined adult population, and little is known about the biological responses to racial stressors in ethnic minority youth. Adolescence is a sensitive developmental stage during which individuals undergo significant changes in biological, cognitive, and socioemotional domains (Berk, 2021) and ethnic health disparities propagate and endure into adulthood (Sanders-Phillips, 2009). During adolescence, youth strive for affiliation with peers and separation from family while continuing their exploration of racial identity (Spear, 2000). Ethnic minority adolescents may be particularly vulnerable to identity-based racial discrimination experiences, which makes it imperative to study social and cognitive factors that can potentially amplify or buffer the detrimental impact of racism. ***Currently, there is neither a study that comprehensively measures ethnic minority adolescents' stress response to subtle racism in relation to their racial discrimination experience nor an investigation that explores the possible risk and protective factors.***

Specific Aim 1

Racism can be differentiated as blatant racism which has hot, close, and direct components (e.g., racial violence, racial slurs), and subtle racism which is cool, distant, and indirect (e.g., racial profiling, bad customer services) (Pettigrew & Meertens, 1995). Both overt and subtle discriminations were distinctively associated with mental health problems and the experience of subtle discrimination was linked to the depressive symptoms (e.g., Noh et al., 2007). Whereas the blatant racism (e.g., racial violence, racial slurs) has been declining, subtle and covert forms of racism still persist (Pettigrew, 1998; Sue et al., 2007). Subtle racism has been evolved into be a major problem in contemporary society, as it often takes the normative and invisible form, and can easily slip out of our conscious awareness (Sue, 2005). Some studies used experimental paradigms, such as retelling stories of someone else being picked on in a store as theft (e.g., Lepore et al., 2006) or overhearing racist comments (Huynh et al., 2017), to induce indirect experience or vicarious exposure to racism. However, no experimental paradigm has been documented to elicit ethnic minority adolescents' direct experience of subtle racism. It is critical to use a carefully designed experimental paradigm to induce a standardized delivery of subtle racism which may commonly occur but easily be overlooked in ethnic minority

adolescents' lives. A laboratory experimental paradigm can also enable the real-time recording of stress responses involving multiple systems.

The stress systems involve hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system (ANS), and inflammatory mediator (IM) functioning (e.g., Weiner, 1992). These systems have been demonstrated to play a unique role in facilitating individuals to meet up the demands of a given stressor and they have important health implications (e.g., Chrousos & Gold, 1992; Levi-Montalcini et al., 1997; Slavish et al., 2015). Specifically, whereas the ANS directs rapid fight-or-flight behavioral responses to threat, the HPA axis facilitates behavioral responses in a sustained fashion. IM supports biological protection and recovery against harmful environmental factors. Due to technical advances, cardiovascular measure of blood pressure, a significant predictor of cardiovascular disease (see Fuchs & Whelton, 2020 for a review), can be monitored automatically to tap on moment-to-moment ANS functioning. Furthermore, stress responses can be measured noninvasively and simultaneously in oral fluids which can be assayed for cortisol, interleukin-6 (IL-6), and c-reactive protein (CRP), commonly used benchmarks for HPA and IM functioning which has demonstrated high validity in predicting health (e.g., Newton et al., 2014; Wang et al., 2016).

Stress response can be captured in terms of stress reactivity and recovery, which are respectively operationalized as the strength of a stress reaction in relation to a baseline and the rate of stress reaction returning to baseline. There are mixed results regarding dysregulated stress reactivity in the literature, as both heightened and blunted stress reactivity have been identified to link with racial discrimination experience (see Busse et al., 2017, for review; Sladek et al., 2021). The discrepant patterns may be due to the timing and duration of racial discriminatory experience that is assessed, whether it being one-time, transient or chronic, on-going. Therefore, a nonlinear relationship between racial discrimination experience and stress reactivity will be tested in the proposed study in that infrequent up to moderate levels of racial discrimination will be correlated with heightened stress reactivity whereas frequent, chronic racial discrimination exceeding moderate levels will be correlated with blunted stress reactivity in response to subtle racism in ethnic minority adolescents. On the other hand, the literature on stress recovery was limited and in general supports that racial discrimination experience is related to slow recovery (e.g., Wagner et al., 2012). It is expected that racial discrimination experience will be associated with slow recovery rate in cortisol, c-reactive protein, and blood pressure returning to baseline after subtle racism. Secondarily, we will include a cognitive stress task to compare whether there will be associations between racial discrimination experience and stress response to cognitive stress.

Specific Aim 2

Although racial discrimination poses significant risks to ethnic minority children's health and stress response, not all children fare out the same. Understanding the interplay of risk and resilience in minority youth population is of paramount importance to the health and well-being of the U.S. population (e.g., Marks et al., 2020). Some social and cognitive factors have been demonstrated to have implications on ethnic minority youths' health outcomes (e.g., Dotterer & James, 2018; Mendoza-Denton et al., 2002; Sellers et al., 2006), however, no studies have examined the roles of those factors as risk or protective factors in moderating the association between racial discrimination and biological stress response to subtle racism among ethnic minority adolescents.

Ethnic minority parents can provide important support to promote their adolescent children's normative and positive development. A growing body of literature has focused on parental ethnic-racial socialization, the practices and messages that parents transmit to children concerning ethnicity and race (e.g., Tran & Lee, 2010). Parental engagement in enculturation in which parents instill in children pride and knowledge about their ethnic groups has been found to mitigate the negative impact of racial discrimination (Dotterer & James, 2018; Hughes et al., 2006; Saleem & Lambert, 2016). On the other hand, preparation for bias, whereby parents discuss exposure to prejudice and discrimination with children, was found to exacerbate the impact of racial discrimination and adolescents' depressive symptoms (Dotterer & James, 2018). It would be meaningful to explore whether specific parental ethnic-racial socialization practices may modify the detrimental effects of racial discrimination experience on ethnic minority adolescents in terms of helping to regulate or dysregulate their biological responses to acute racial stressors.

How ethnic minority youth interpret racial stressors, particularly subtle discrimination, can have important implications in their stress responses. Cognitive appraisal of subtle discrimination, not overt discrimination, has been found to mediate the effect of discrimination on depressive symptoms in adult immigrants (Noh et al., 2007). Due to their history of mistreatment and discrimination, ethnic minority youth tend to interpret negative interpersonal interactions as motivated by discrimination or racial bias (Feldman Barrett, & Swim, 1998; Mendoza-Denton et al., 2002), which may elicit high stress and arousal to regulate. For example, African American women in midlife who

attributed mistreatment in daily life to racial discrimination exhibited heightened blood pressure reactivity particularly in response to acute racial stressor (Guyll et al., 2001). Although plausible, there is no evidence regarding the exacerbating effect of ethnic minority adolescents' racial attribution in relation to their stress responses to subtle racism.

Another important cognitive factor is ethnic minority adolescents' racial identity. Two important dimensions of racial identity include centrality and positivity, the extent to which race is perceived as important to self-concept and regarded as positive (Sellers et al., 1997). It has been proposed that racial identity can mitigate the negative effects of racial discrimination (Gaylord-Harden et al., 2012). Although the findings regarding the role of racial identity on psychological distress were mixed (see Brondolo et al., 2009 for a review), some studies have found that being strongly identified as a member of one's ethnicity and holding positive views about one's ethnicity buffered against the deleterious effect of racially based stressors (e.g., Brody et al., 2015; Sellers et al., 2006; Wong et al., 2003). A recent study revealed the relationship of perceived discrimination experience with greater HPA axis reactivity during the social-evaluative task in African American adolescents with low racial identity but with greater inflammatory response over the recovery phase among those with strong racial identity (Lucas et al., 2017). This suggests the stress-buffering benefit of strong positive racial identities in attenuating stress reactivity and facilitating recovery among ethnic minority adolescents.

Based on previous studies, it is expected that parental ethnic-racial socialization, ethnic minority adolescents' racial attribution and racial identity will moderate the relationship between their racial discrimination experience and dysregulated stress response to subtle discrimination. It is expected that the associations between experience of racial discrimination and dysregulated stress response (i.e., heightened/blunted stress response, slow recovery) will be weaker among adolescents whose parents engage in parental enculturation and those who have strong ethnic/racial identity. It is also expected that the associations will be stronger among adolescents whose parents promote mistrust and those who make attribution to racism.

2. Approach

The PI proposes a study that will investigate the relationship between racial discrimination experiences and ethnic minority adolescents' dysregulated responses to subtle racism as well as cognitive stress. We will assess ethnic minority adolescents' reactivity to and recovery from the subtle racism by employing an experimental paradigm. Adolescents' cardiovascular and neuroendocrine-immune responses will be assessed before, during, and after the peer rejection condition which has been carefully designed to elicit subtle racism, rejection from White peers. We will also measure parental racial socialization, adolescents' racial attribution, and racial identity through self-report questionnaires.

Participants

A community sample of 90 ethnic minority adolescents (45 Black/African American, 45 Latino American) ages 9-14 and their parents will be recruited from Bowling Green, KY area. Study information and flyers will be distributed to local communities, at family events, and through word of mouth. Two community helpers/translators from local ethnic communities will be hired to help recruit and schedule participants for lab visits.

After completing the first lab visit, adolescents' report of peer victimization experience and social anxiety disorder severity scale, and responses to questionnaires on racial discrimination experience, depression, anxiety, and social anxiety will be used as screening measures for the second lab visit. Adolescents who respond to any peer victimizing item with response of "most day" "every day" (Sweeting, Young, West, & Der, 2006), had high frequency of racial discrimination experience, or had severely high scores on depressive symptoms, social anxiety disorders, and general anxiety disorders will not continue with the second lab visit. Parents of those excluded adolescents will be informed of the exclusion because of the concern that the child may be triggered by the peer rejection task during the second lab visit. Parents will also be encouraged to contact Bowling Green Independent School District student and family counselor or Warren County public school guidance department to get professional counseling service.

Procedure

Parents and adolescent children will have two lab visits, roughly two weeks apart. During the first visit, adolescents will provide consent and assent to participate in the study. Adolescents will provide initial information to be used during the peer rejection task in the second visit and complete questionnaires on racial discrimination experience and their racial identity. To assess health conditions that may have confounding impact on stress responses, adolescents

will also report mental health problems (e.g., anxiety), physical health problems (e.g., diabetes), medication history, and oral health conditions (e.g., gum disease). Parents will complete questionnaires to provide demographic information, and report their ethnic-racial socialization practices and adolescents' experience of negative life events. Adolescents will complete a cognitive stress paradigm which involves a 5-minute resting period of watching a silent nature video clip, a 5-minute impossible puzzle task, and a 15-minute recovery period. During the recovery period, adolescents will complete a set of questionnaires to rate their emotions and attributions about the impossible puzzle task and then read animal magazines.

At the second study visit, adolescents will go through the subtle racism induction paradigm involving a 5-minute resting period of watching a silent nature video, a 5-minute peer rejection task, and two recovery periods. During the first 15 minutes of recovery, they will complete a set of questionnaires to rate on their emotions and attributions about the game, as well as read an animal magazine. In the following 30 minutes, they will be told to continue reading some more animal magazines until the whole paradigm ends. Adolescents will provide five saliva samples right before the peer rejection condition starts, as well as 5, 15, 30, and 45 minutes later. The timing of collecting salivary samples follows the common procedure used in other studies (e.g., Lucas et al., 2017), as stress response in saliva peaks around 20 minutes after the onset of the stressor and then declines (Gunnar et al., 2009). At the end of the second lab visit, adolescents will be invited to go through additional measures (i.e., parent-child conversation, peer inclusion condition) to offset any distress after the peer rejection task. In the end, adolescents and their parents will be debriefed, thanked, and received \$80 as compensation. To control for diurnal rhythm in cortisol, second lab visits will be scheduled between 2pm and 7pm.

Measures

The Impossible Puzzle Task

To elicit cognitive stress, adolescents will be asked to complete an impossible puzzle task which lasts for 5 minutes. They are told to try to solve the puzzle by fitting all pieces back in the wooden frame, however, with one wrong piece provided.

The Peer Rejection Task

To elicit the experience of subtle racial discrimination, adolescents will complete an adapted peer rejection task (e.g., Silk et al., 2012; McQuade & Breau, 2017). The task has been demonstrated as effective in manipulating peer rejection (Silk et al., 2012) and can elicit increased physiological reactivity (e.g., McQuade & Breau, 2017). During the first visit, adolescents will provide initial information to help set up the social rejection task during the second visit. They will answer questions about their sex, age, and have their picture taken to create a profile for the social rejection task. Then adolescents will be provided with photos and profiles of 6 age- and gender-matched fictitious peers and select 4 of them that they will be most interested in chatting with.

During the second visit, they will be informed that they have been matched with 2 of the peers they select during their first visit and the peers will take turns to choose who they would like to chat with about 16 different topics (e.g., friends; music). To manipulate peer rejection, the task is programmed so that the participant is not chosen for 75% of the time. A large X appears on the picture of the player who is not chosen. Each round has 16 trials and the whole peer rejection condition will last about 6 minutes.

Here below is the screen shot of the peer rejection condition on the computer. During the game, the participant's picture is shown on the upper left, the photos on the bottom left and upper right represent fictitious peers. The prompt of "(Peer's name), who would you rather talk to about ____" appears besides the fictitious peer on the bottom left who will make a choice. The selecting peer chooses the participant (acceptance) or the other fictitious peer (rejection) to discuss topics of teen interest (e.g., movies).



Figure 2. Screenshot of the Peer Rejection Condition

After each round of peer rejection trials, adolescents will be asked whether they would like to continue and be reminded that nobody will be mad if they decide to stop. To offset the distress adolescents may have after the peer rejection task, a set of measures will be taken at the end of the second lab visit. First, adolescents and their parents will be invited to talk about dinner time and the peer rejection task. Adolescents will also be invited to go through the peer inclusion condition whereby they will be invited to play the interaction task with 2 other peers they select during the first lab visit and they will be picked by the peers to have chat with 75% of the time. At the end of the second last visit, adolescents and parents will be debriefed that the peers and their profiles are not real and there is a preset computer program that decides who will get picked in the task. They will be shown the computer program that has been used to create the task and encouraged to ask any questions they may have. Parents will be suggested to use counseling service at Western Kentucky University if they observe any excessive distress from their child afterwards. The experimenter will stop the study if the adolescent displays any upset, anxiety, or distress during the task.

The Parent-Child Conversation about the Peer Rejection Task

Right after the peer rejection task, the dyads were asked to talk about last night's dinner for 2.5 minutes and then the peer exclusion task for 5 minutes to offset the distress. Parents were provided with a list of suggested question prompts on the causes, thoughts and emotions, and strategies regarding the peer rejection task to guide their conversations with children. Parents were also told to adapt or skip the question prompts in the ways they were comfortable with.

Biological stress response to the impossible puzzle task

During the first lab visit, adolescents' various biological responses will be recorded. Adolescents will be connected to a blood pressure monitor and a biolog, with which their diastolic blood pressure (DBP), systolic blood pressure (SBP), skin conductance (SC), and respiratory sinus arrhythmia (RSA) will be measured continuously throughout the resting period, task period, and recovery period.

Biological stress response to the peer rejection task

During the second lab visit, adolescents' various biological responses will be recorded. Adolescents will be connected to a blood pressure monitor and a biolog, with which DBP, SBP, SC, and RSA will be measured continuously throughout the resting period, task period, and two recovery periods.

Five Salivary samples will be collected from each adolescent. Adolescents will drink 2.5ml of water upon arrival at the laboratory, as well as after each salivary collection. Participants will be asked to refrain from consuming food, caffeine, citric drinks and dairy, to avoid exercise or brushing teeth in the 30 minutes prior to saliva collection, and to report adherence to these guidelines. Participants will provide 2 mls whole saliva by passive drool at each time point. Saliva samples will be divided into approximately 1 mL aliquots to minimize the impact of freeze-thaw cycles on salivary analyte data. Aliquoted samples will be stored at -20 C until shipped frozen overnight to Salimetrics laboratories (State College, PA). Samples will be assayed for cortisol (HPA axis markers), and IL-6 and CRP (inflammatory/immune markers) at each of the five collection timepoints for all participants.

Biological stress response to the parent-child conversation about the peer rejection task

During the second lab visit, adolescents' various biological responses to the parent-child conversations about last night's dinner and peer rejection task will be recorded. Adolescents will be connected to a blood pressure monitor and a biolog, with which their DBP, SBP, SC, and RSA will be measured continuously throughout the baseline period of dinner conversation and the task period of discussing peer rejection task.

Racial discrimination experience

Racial discrimination will be assessed using the Adolescent Discrimination Distress Index (ADDI; Fisher et al., 2000). The ADDI is one of the most widely used measures of discrimination which captures interpersonal discrimination in daily life. Adolescents will rate on how often they are treated unfairly due to their race or ethnicity in the past year on a scale from 1 (never) to 5 (more than 10 times). A composite average across all 15 items will be used in analysis with higher scores indicating great levels of perceived racial discrimination.

Experience of negative life events

Parents will report the adolescents' experience of negative life events (e.g., moved house) in the past year by completing Child and Adolescent Survey of Experiences (CASE-P; Allen et al., 2011). Parents will indicate whether each of events has occurred in the previous 12 months and rate its impact on child's life on a 6-point scale from 1 (really good) to 6 (really bad).

Parental ethnic-racial socialization

Parents' racial socialization will be assessed using the self-report measure developed by Hughes & Johnson (2001). The measure contains 16 items tapping on the frequency of different types of racial socialization practices, including enculturation, preparation for bias, promotion of mistrust. Items will be rated on a 5-point Likert scale ranging from 1 (never) to 5 (10 or more times).

Racial identity

A modified version of the Multidimensional Inventory of Black Identity-Teen (MIBI-t; Scottham et al., 2008) will be used to assess adolescents' ethnic/racial identity. "Black" in the original questionnaire will be replaced with "your ethnic group" in the modified questionnaire. Adolescents will rate on a 5-point liker scale from 1 (strongly disagree) to 5 (strongly agree) to rate how much they agree with each item. Responses to 9 items tapping on centrality and positivity will be averaged and used.

Adolescents' attribution about subtle racism task

Adolescents will rate on the likelihood of a set of reasons to explain the unfair treatment in the peer rejection task. The reasons are developed based on previous studies (e.g., Liao et al., 2016), including attribution to racism (e.g., "I am from a different ethnic group"; "the other players are racist"), chance, ineffective communication, and etc. Responses regarding attribution to racism will be summed and used in the analysis.

Adolescents' attribution about cognitive stress task

Adolescents will rate on the likelihood of a set of reasons to explain why they did not solve the puzzle. The reasons are developed based on previous studies and included intrinsic (e.g., "I am not smart") and extrinsic attribution (e.g., "I ran out of time"). Responses regarding intrinsic attribution will be summed separately.

Adolescents' emotions regarding subtle racism task

Adolescents will report the intensity of five negative emotions that they have experienced during the peer rejection task. Ratings for the negative emotions will be summed.

Adolescents' emotions regarding cognitive stress task

Adolescents will report the intensity of five negative emotions that they have experienced during the peer rejection task. Ratings for the negative emotions will be summed.

3. Data Analysis Plan

Manipulation of the impossible puzzle and peer rejection task will be checked through adolescents' ratings of emotions and attributions for each task.

Hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002) will be used for analysis. This modeling technique can accommodate the nested nature of the dataset, that is, time points of measuring stress responses are nested within each individual. It is also advantageous in handling missing value points with multiple imputations. HLM splits variability into within-person (Level 1) and between-person (Level 2) components. At Level 1, each adolescent's stress response trajectories at different time point will be modeled, with intercept representing baseline, a time variable representing linear change of stress response (i.e., reactivity), and time² variable representing quadratic change of stress response (i.e., recovery). Y_{10} will be significantly positive and Y_{20} will be significantly negative to indicate a significant reactivity and

recovery in stress response to subtle racism. At Level 2, adolescents' personal variables will be entered to explain the level 1 trajectory variables. Here below is the base model.

The base model

Level 1 (within-child): $\text{Cortisol} = B_{t0} + B_{t1} \times \text{time} + B_{t2} \times (\text{time}^2) + \text{error}$

Level 2 (between-child): $B_{t0} = Y_{00} + \text{error}$

$B_{t1} = Y_{10} + \text{error}$

$B_{t2} = Y_{20} + \text{error}$

Specific Aim 1:

To examine Aim 1, racial discrimination and racial discrimination² will be entered at level 2 to model linear and nonlinear effects of racial discrimination on stress reactivity. It is expected that Y_{11} will be significantly positive if a linear relationship between racial discrimination and stress reactivity exists, and Y_{12} will be significantly negative if a nonlinear relationship exists. Racial discrimination will be entered at Level 2 to model recovery. Given the hypothesis, Y_{21} will be significantly positive if racial discrimination is associated with slower recovery. Adolescents' experience of negative life events, age, gender, body mass index (BMI), and parents' education levels will be included as control variables if they are significant in the model and will be dropped if not.

Level 2 (between-child): $B_{t0} = Y_{00} + \text{error}$

$B_{t1} = Y_{10} + Y_{11} \times \text{racial discrimination} + Y_{12} \times \text{racial discrimination}^2 + Y_{13} \times \text{negative life event} + Y_{14} \times \text{age} + Y_{15} \times \text{gender} + Y_{16} \times \text{BMI} + \text{error}$

$B_{t2} = Y_{20} + Y_{21} \times \text{racial discrimination} + Y_{22} \times \text{negative life event} + Y_{23} \times \text{age} + Y_{24} \times \text{gender} + Y_{25} \times \text{BMI} + \text{error}$

Specific Aim 2

To examine Aim 2, separate level 2 models predicted with each moderating variable (i.e., parental enculturation or promotion of mistrust, adolescents' racial attribution and their racial identity centrality) pertaining to the peer rejection task will be constructed.

The moderating variable and its interaction with racial discrimination and racial discrimination² will be added in predicting reactivity and recovery. Y_{14} or Y_{15} is expected to be significant if the potential risk/protective factor moderates the relationship between racial discrimination and stress reactivity. Y_{24} is expected to be significant if the potential risk/protective factor moderates the relationship between racial discrimination and stress recovery. Post-hoc analyses will be conducted to examine how those relationships differ between adolescents those are at high (1 SD above the mean) and low (1 SD below the mean) levels of the moderating factors. Adolescents' experience of negative life events, age, gender, BMI, and parents' education levels will be included as control variables if they are significant in the model and will be dropped if not.

Level 2 (between-child): $B_{t0} = Y_{00} + \text{error}$

$B_{t1} = Y_{10} + Y_{11} \times \text{racial discrimination} + Y_{12} \times \text{racial discrimination}^2 + Y_{13} \times \text{parental enculturation (promotion of mistrust/racial attribution/strong positive racial identity)} + Y_{14} \times \text{racial discrimination} \times \text{parental enculturation (promotion of mistrust/racial attribution/ strong positive racial identity)} + Y_{15} \times \text{racial discrimination}^2 \times \text{parental enculturation (promotion of mistrust/racial attribution/ strong positive racial identity)} + Y_{16} \times \text{life stressor} + Y_{17} \times \text{age} + Y_{18} \times \text{gender} + Y_{19} \times \text{BMI} + \text{error}$

$B_{t2} = Y_{20} + Y_{21} \times \text{racial discrimination} + Y_{22} \times \text{parental enculturation (promotion of mistrust/racial attribution/ strong positive racial identity)} + Y_{23} \times \text{racial discrimination} \times \text{parental enculturation (promotion of mistrust/racial attribution/ strong positive racial identity)} + Y_{24} \times \text{life stressor} + Y_{25} \times \text{age} + Y_{26} \times \text{gender} + Y_{27} \times \text{BMI} + \text{error}$

Alternative data analysis plan

This approach starts with calculating summative measure for biological stress response. Following a well-established area under the curve (AUC) method (Pruessner et al., 2003), the summative score is the trapezoid area

summarizing changes across timepoints for each individual biological measurement. The method is developed specifically for use in biological reactivity paradigms which involve multiple points of measurement with different time distances between measurements. AUC during the stress reactivity period (Area S1 in Figure 4) will be calculated for stress reactivity. For SBP, DBP, SL, and RSA reactivity, the duration of the impossible puzzle or the peer rejection condition (5 minutes) will be used as the stress reactivity period; for cortisol and IL-6 reactivity (only assessed for the peer rejection task), due to their delayed response, the time period between 1st and 3rd sample will be used; for CRP (only assessed for the peer rejection task) with the longest delayed response (Lucas et al., 2017), the time period between 1st and 4th sample will be used. AUC during the stress recovery period (Area R1 in Figure 4) will be calculated and the composite score of Area R2/(Area R1+Area R2) will be used to indicate stress recovery (Kario et al., 2002). For SBP, DBP, SC, and RSA recovery, the stress recovery period will be the first 15-minute period following the end of the impossible puzzle task period and 45-minute period following the peer rejection task. The stress recovery period will be between 3rd and 5th sample for cortisol and IL-6, and between 4th and 5th sample for CRP.

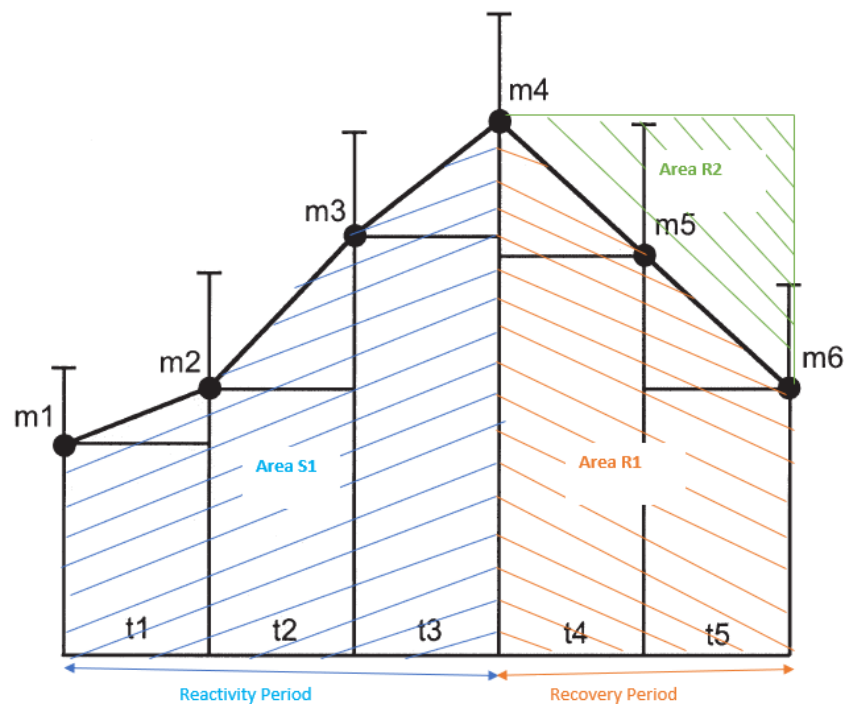


Figure 4 Illustration of Stress Response Across Time Points
(Reactivity Period and Recovery Period Vary for different biological measure)

Specific Aim 1:

Separate regression analyses will be constructed for predicting stress reactivity or recovery of each biological measure (i.e., DBP, SBP, cortisol, IL-6, and CRP). For models predicting stress reactivity, both linear and nonlinear quadratic effects of racial discrimination (i.e., racial discrimination, racial discrimination²) entered as the predictor variables. For models predicting stress recovery, only racial discrimination will be used as the predictor variable. Adolescents' experience of negative life events, age, gender, BMI, and parents' education levels will be included as control variables if they are significantly correlated with the outcome variable. It is expected that coefficient for racial discrimination or racial discrimination² will be significant if a linear or nonlinear relationship between racial discrimination and stress response exists.

Specific Aim 2:

To test aim 2, each potential risk/protective factor (i.e., parental enculturation/parental promotion of mistrust/racial attribution/ strong positive racial identity), and its interaction terms with racial discrimination and the quadratic term of racial discrimination (racial discrimination²) will be added to regression models pertaining to the peer rejection task. It is expected that coefficient for the interaction term will be significant if the potential risk/protective factor moderated the relationship between racial discrimination and stress response. Post-hoc analyses will be conducted to examine how those relationships differ between adolescents those are at high (1 SD above the mean) and low (1 SD below the mean) levels of the moderating factors.

Power analysis

There is no consensus standard for power analysis regarding hierarchical linear modeling (Castelloe & O'Brien, 2000), as covariance structure in determining HLM power level is unknown until collecting data (Fang, 2006). Prior studies have demonstrated that small sample sizes in level 1 and level 2 have little biasing effect for fixed effect estimate (e.g., Bell et al., 2009). Some general rules of thumb with Monte Carlo simulation recommend level 1 sample size up to 30 and level 2 sample size up to 200 for detecting medium and large fixed effects and level 2 sample size bigger than 200 for detecting interaction effect (Arend & Schafer, 2019). But it is generally suggested that any preliminary power analysis for HLM is an educated guess and researchers need to be flexible and practical in determining the sample size using hierarchical linear modeling. With regard to the alternative data analysis plan, G*Power 3.1 is used to determine the sample size (Faul et al., 2008). For prior sample size analysis on R² deviation from zero in linear multiple regression with adequate power (.80) with 5 predictors, a sample of 43 is suggested to detect a large effect size ($f^2 = 0.35$) and a sample of 92 to detect a medium effect size ($f^2 = 0.15$). A sample of 80 in the proposed study may have enough power to detecting a medium to large effect size.