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Biopsychosocial predictors of testosterone and its reactivity to acute psychosocial stress in adolescents

THESIS

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MASTER OF ARTS

in Social Ecology

by

Emma Louise Rodgers

Thesis Committee:
Professor Kate Ryan Kuhlman, Chair
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DEDICATION

To

the generations of researchers who applied their lives to answering questions that are, themselves, inherently without answer; to all those who still bravely attempt to quantify the immeasurable aspects of what it means to be human.

We are all storytellers.

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ACKNOWLEDGEMENTS

It is evident to me that my successes have arrived by way of gift. I cannot entirely own my accomplishments, for as any collaborative scientist knows, accomplishments are not possible without input from a variety of sources—both personal and professional. The joy of this moment is a direct reflection of the support I have received from members of my research families, which began in a basement in Detroit, and continue today in a conference room overlooking a distant mountain range.

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ABSTRACT OF THE THESIS

Biopsychosocial predictors of testosterone and its reactivity to acute psychosocial stress in adolescents

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Emma Louise Rodgers

Master of Arts in Social Ecology

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Professor Kate Ryan Kuhlman, Chair

Few studies investigate the impact of psychosocial stress on acute testosterone activity in adolescents, despite theoretical understandings that testosterone's relationship with aggressive behavior is dependent upon stress or challenge contexts. Effective administration of laboratory stress, biospecimen collection and storage protocol, and testosterone assaying processes are all important methodological considerations that may explain why the link between testosterone and acute stress response remains unclear in this population. Additionally, the biopsychosocial factors that may predict acute testosterone reactivity in this population also remains unknown. As such, the current study addresses these gaps by examining testosterone activity (via salivary samples) across the administration of a Trier Social Stress Test in a sample of 88 adolescents (46.4% female, M_{age} = 13.91 years, SD_{age} = 1.57). A series of nine biopsychosocial factors (age, gender,

pubertal status, affective reactivity, prosocial behaviors, conduct problems, emotional and behavioral regulation, and exposure to early life adversity) were examined as potential predictors of (1) overall testosterone levels and (2) acute changes of testosterone across the administration of laboratory stress. Results indicated a robust response to psychosocial stress, supporting the notion that testosterone mounts an acute stress response in adolescents. However, only age and sex uniquely predicted overall testosterone and acute reactivity to stress in this sample. These findings suggest a methodological framework for capturing testosterone reactivity to acute stress, and provide evidence for future research to clarify the role of testosterone reactivity in adolescent behavior.

INTRODUCTION

The gonadal hormone testosterone has been implicated in dimensions of behavior and health across the lifespan (e.g. relationship quality, social status, metabolic and cardiac functioning) (Booth et al., 2006; Edelstein et al., 2014; Kelly & Jones, 2013), with a notably important role during adolescence. Produced by the testes, ovaries, and, in small doses, the adrenal glands, testosterone increases at the onset of puberty. In conjunction with other hormones, testosterone facilitates sexual maturity, including the development of secondary sex characteristics, in both males and females (Peper et al., 2010). In addition to its role in sexual development, testosterone has been heavily implicated in aspects of neurological development (e.g. white matter volume, reward circuitry) (Op de Macks et al., 2011; Perrin et al., 2008) and social development (e.g. dominance behaviors) (Vermeersch et al., 2010) during this time period, underscoring its role across many domains of adolescent functioning.

Aggression is perhaps the most popular behavioral correlate of testosterone (Geniole et al., 2020), and is a problem behavior that can have steep repercussions that extend into adulthood if it is present during adolescence (Poulton et al., 2015). Considering a wealth of animal models directly linking levels of testosterone to aggressive behaviors (Giammanco et al., 2005), as well as findings from adult males who engage in more severe forms of these behaviors (e.g. violent offenders) (Dabbs et al., 1987), researchers have been eager to explore the connections between testosterone and aggression in adolescents. However, findings connecting overall testosterone levels and problem behaviors during adolescence have been mixed (e.g. Duke et al., 2014), suggesting the need for a more nuanced approach to investigating these associations in this developmental period.

Notably, there is growing theoretical and empirical evidence to suggest that including overall testosterone levels alone may not be adequate when exploring pathways to adolescent problem behaviors (Geniole et al., 2020). First, testosterone typically increases at the *beginning* of puberty—ages 9-11 for females, ages 10-12 for males—as the hypothalamus-pituitary-gonadal (HPG) axis activates to spur physical growth and reproductive maturity (Peper & Dahl, 2013). As such, by the time adolescents reach the mid-teenaged years, they have most likely already experienced the greatest increase of testosterone in their development. Were overall testosterone levels the sole cause of problem behaviors, we would expect a rise in problem behaviors occurring during the preteen years—far earlier than these trends play out in vivo (Poulton et al., 2015). Further, testosterone levels decrease steadily across the lifespan following adolescence (Harden et al., 2016), which does not account for the general decrease in delinquency and aggressive behaviors that are observed during the transition to adulthood. Considered together, these mismatches suggest that the key to understanding the relationship between testosterone and behavior during adolescence may lie in more nuanced markers of functioning. In other words, overall testosterone may not predict behaviors like aggression, but rather, how testosterone operates within specific contexts.

One promising consideration involves testosterone reactivity to stressors. Growing evidence suggests that the testosterone-aggression link in humans is influenced heavily by contexts of acute stress (Carré & Archer, 2018). Testosterone is produced following activation of the HPG axis, which reacts to stress alongside the hypothalamus-pituitary-adrenal axis (Toufexis et al., 2014). Moreover, testosterone may be more directly associated with social-dominance behaviors that can provoke aggression when coupled

with environmental stressors (Archer, 2006). This is known as the Challenge Hypothesis, which posits testosterone's impact on aggression operates through the context of social threat or challenge. However, whether and by what mechanism levels of adolescent testosterone respond to acute stressors is largely unknown, despite early findings indicating this as a promising direction (Popma et al., 2007). Considered together, exploring testosterone activity in the context of stress is necessary to address the complexity of its role in problem behaviors in adolescence. Given the steep potential consequences that adolescents face for such behavior, including social dysfunction (Geniole et al., 2020) and, in extreme cases, involvement in the criminal justice system, it is crucial to explore the unique functioning and developmental underpinnings of multiple indices of testosterone activity in this demographic.

Measuring testosterone and testosterone responses to acute stress.

Psychological research has explored the role of testosterone in human behavior for over 50 years; the earliest study directly investigating the association between aggression and testosterone was conducted in 1971 (Persky et al., 1971). Over the next several decades, technological advances in testosterone sample collection and analysis have revealed many important methodological considerations (Trost & Mulhall, 2016). For example, many studies measure testosterone via saliva samples collected at between one and three instances (Aronoff & DeCaro, 2019; Klimas et al., 2019). Additionally, some experimental studies utilize a single pre-post sample collection approach (van der Meij et al., 2010). Such designs are typically cost-effective and useful in determining overall

testosterone levels, but do not allow for more acute measures of testosterone activity that may provide more nuanced insight into how testosterone influences behavior.

As a result, while the HPG axis has been shown to respond to stress broadly (Toufexis et al., 2014), specific patterns of testosterone's stress response have not been well defined, particularly among adolescent populations. Study designs utilizing standardized laboratory stressors may be integral to defining patterns of stress reactivity. Animal models examining the impacts of stress on testosterone have revealed a reliable increase in acute testosterone levels following a standardized stressor (e.g. novel environments, Lürzel et al., 2010), providing evidence to support the use of similar designs in human adolescent populations. The Trier Social Stress Test (TSST) is a well-replicated standard of inducing acute psychosocial stress in humans in the laboratory (Birkett, 2011). The TSST involves elements of judged public speaking and public arithmetic that, coupled with an absence of positive feedback, is reliable at eliciting stress responses. The TSST may be particularly impactful for adolescents, who are far more sensitive to psychosocial stressors than individuals at other developmental timepoints (Buwalda et al., 2011).

Previous research has explored the impacts of the TSST on an array of biomarkers, including cortisol, heart rate, and skin conductance (Goodman et al., 2017; Hellhammer & Schubert, 2012; Pisanski et al., 2018). Studies conducting in-depth investigations into the TSST's impact on functional markers of the HPG-axis, however, remain comparatively few and have yielded mixed results. Some findings indicate that acute testosterone levels do not respond to the TSST (Schoofs & Wolf, 2011), while others reveal a significant increase in testosterone levels following the administration of the TSST (Bernhard et al., 2021;

Lennartsson et al., 2012). A potential source of these discrepancies may lie in methodological inconsistencies. For example, a TSST administered in an unstandardized fashion may not be adequate in eliciting a stress response, and too few biospecimen collection points could fail to capture an acute response. Based on previous findings, studies utilizing multiple pre-TSST collection timepoints (i.e., those which establish an adequate baseline) in conjunction with multiple post-TSST collections yield results that more strongly indicate an acute testosterone response (Bedgood et al., 2014; Lennartsson et al., 2012). Other important considerations include the standardization of specimen collection protocol across examiners, adequate storage for maintaining biospecimen validity (namely, specimen temperature and labelling standards), and testosterone assaying processes (Granger et al., 2004). Considered together, these findings support the notion that methodology may be the key to understanding acute testosterone activity, yet a design which follows these parameters has not been conducted in an adolescent sample. As a result, many characteristics of acute testosterone activity remain entirely unknown, including whether patterns of testosterone reactivity can be predicted by specific biopsychosocial factors.

Predictors of testosterone reactivity in adolescence.

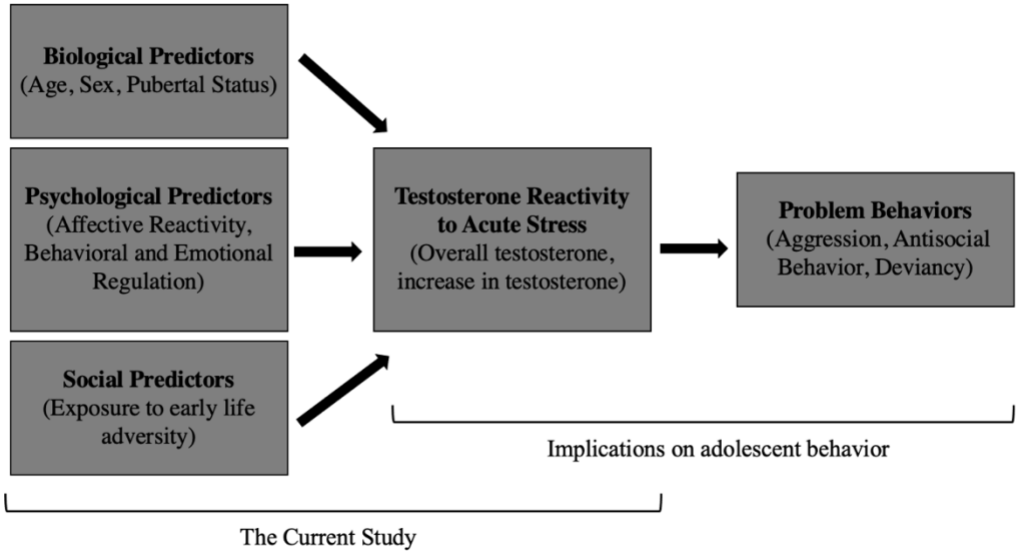
A wealth of literature has investigated potential factors associated with testosterone over the past several decades. The most well-established biological correlates of overall testosterone are sex, age, and pubertal status (Handelsman et al., 2016; Nottelmann et al., 1987), and it is best practice for any investigations into adolescent testosterone to consider the role of these constructs. Less is known about whether these factors influence

testosterone reactivity to acute laboratory stress. Other models have implicated testosterone in processes of emotion regulation (Wu et al., 2018) and behavior regulation (Nguyen et al., 2017). Similarly, systems involved in affective reactivity have been examined in the context of testosterone (Vijayakumar et al., 2019). Among social factors, exposure to early life stress has been linked with later testosterone patterns in both human and animal models (Belsky, 2019; Eck et al., 2020; Zito et al., 2017).

One unifying thread connects nearly all these studies—a study design that includes only a single index of testosterone. Indeed, virtually all previous explorations in this area have operationalized testosterone using a variation on total testosterone levels. Whether and how these associations may differ when considering multiple markers of testosterone response to acute stress remains unknown.

The current study aimed to address the mixed findings surrounding testosterone activity during adolescence. Specifically, it attempted to establish a methodological framework for expanding testosterone analyses to include two indices of activity (overall testosterone levels and testosterone reactivity to acute stress) by exploring the impact of a highly standardized laboratory stressor across multiple timepoints of testosterone collection. Further, we investigated the impact of a series of biopsychosocial predictors on the two indices of testosterone activity to determine the presence and nature of any observed differences. Finally, we attempted to codify a conceptual framework for future studies investigating the relationship between testosterone and problem behaviors during adolescence (Figure 1). To our knowledge, this is the first study of its kind to explore unique predictors of these indices side-by-side in an adolescent sample.

Figure 1. Conceptual framework of the current study.



METHODS

Participants.

The current study included 88 adolescents (46.4% female, M_{age} = 13.91, SD_{age} = 1.57) of diverse ethnic and racial backgrounds (26.8% identified as Hispanic/Latinx, 72.2% as White, 17.5% as Asian, 10.3% as Black/African American, 3.1% as Native Hawaiian or Other Pacific Islander, and 2.1% as American Indian or Alaska Native, with some adolescents reporting that they identify with multiple categories). Participants were part of the Teen Resilience Project (TRP), a larger project aimed at investigating early life adversity, inflammatory reactivity in response to acute psychosocial stress, and their associations with adolescent mood and behavior (Kuhlman et al., 2022). Families were recruited to participate in TRP via mass-mailing to households with children aged 12-15 in the Southern California area. Adolescent eligibility was determined following a brief phone interview with the parent. Adolescents who were not fluent in English, had histories of major depression, autism spectrum disorder, psychotic symptoms or mania, and who had current chronic illnesses or medical conditions which could impact biospecimen collection were not eligible to participate.

The overall TRP sample contained 97 adolescents. However, only 88 (90.7% of overall sample) had complete testosterone data and were included in current analyses. The 88 adolescents in the current sample did not differ significantly from those without full testosterone data on either age [$t(86) = .49$, $p = .63$] or sex [$t(86) = .82$, $p = .23$].

Study procedures.

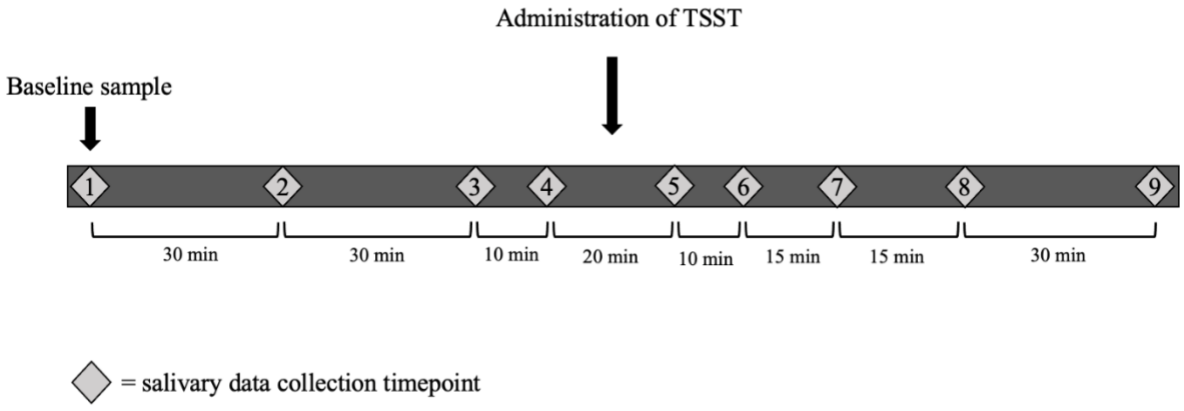
The Institutional Review Boards at the University of California Irvine and the University of California Los Angeles approved all study protocol and procedures. Data were collected during a single laboratory study visit lasting approximately three hours. All study visits began between the hours of 1-4 PM, in consideration of the potential interference caused by diurnal hormone patterns (Granger et al., 2004). Adolescents and their parents completed written informed assent or consent prior to the beginning of the study. Adolescents and their parents were also asked to complete a series of questionnaires containing demographic questions and reports of adolescent mood, behavior, and functioning. Adolescent participants were compensated $60 dollars for completion of study visit day activities.

Testosterone samples were collected via passive drool into sterile salivettes; adolescent sample collection was overseen by study examiners. In total, nine salivary testosterone samples (T1-T9) were acquired over the course of the visit (see Figure 2). Data collection occurred at four timepoints before (-70, -40, -10, 0 minutes) and five timepoints following (+5, +15, +30, +45, and +75 minutes) a laboratory stressor. Upon collection, all saliva samples were labelled, immediately frozen at -20° C, and stored for analysis.

Adolescents underwent an acute psychosocial stressor between samples T4 and T5 (approx. 70 minutes after their arrival to the lab). The Trier Social Stress Test for Children (TSST-C) was administered to all participants. The TSST has been found to produce a reliable stress response in laboratory settings, and the current study followed its exact administrative protocol as outlined by Buske-Kirschbaum and colleauges (1997).

Participants were asked to prepare and deliver an impromptu speech and complete challenging verbal arithmetic problems in front of a panel of two judges. To successfully elicit a stress response, all judges underwent training during which they were given instructions to not provide participants with any verbal or nonverbal cues of support while adolescents were completing this task.

Figure 2. Teen Resilience Project (TRP) salivary data collection timeline.



Analyzing testosterone samples.

To assess testosterone levels from saliva samples, a Salimetrics brand enzyme-linked immunosorbent assay (ELISA) kit was utilized. Salimetrics kits are intended for analyzing testosterone levels in research settings, and all testing protocols from the manufacturer's instructions were followed without alteration.

Specifically, the kits consisted of a 96-well microtiter plate. Wells are coated with a testosterone-binding antibody base, to which testosterone in the form of standards,

controls, and salivary samples are introduced in 25µ amounts to compete for binding sites. Plates are incubated and shaken for 1 hour while binding occurs. Then, plates are rinsed four times before the substrate tetramethylbenzidine (TMB) is introduced. TMB reacts within the bound testosterone, producing a blue color that corresponds with the amount of testosterone that bonded with the antibody base in each well. TMB is then incubated in darkness for 30 minutes. After incubation, 2M Sulfuric Acid is introduced to each well to stop the reaction, resulting in a yellow hue that is analyzed using a PowerWave HT (BioTek Instruments) spectrophotometer, at 450nm. Levels of testosterone in participant samples are calculated by comparing participant samples to a standard curve generated by the controls and samples on each plate. Generating an individual standard curve on each plate helps researchers account for potential inconsistency between plates and is generally considered best practice in biospecimen analysis.

Each participant's nine samples were tested, for a total of 792 assays across participants with available saliva. Information regarding sample abnormalities, including color or high mucin content, were noted for consideration before testing. Of note, testosterone was third in a series of seven salivary biomarkers, including markers of inflammation (e.g. interleukin-6, C-reactive protein) and general biological reactivity (e.g. cortisol), to be analyzed from the samples collected during TRP. As such, in some rare cases (n = 14), there was an insufficient amount (25µ) of sample to test. One participant displayed abnormally high testosterone levels at multiple timepoints (ex: M_{T4} = 8901.1 pg/mL, compared with overall sample M_{T4} = 72.33 pg/mL) that could not be explained with available information. Further, the re-processing of this participant's samples using dilution protocols did not yield different results. As such, this participant's data were

excluded from overall analyses. All testing processes took place at the Institute for Interdisciplinary Salivary Bioscience at the University of California, Irvine.

Calculating testosterone reactivity.

To effectively capture both total testosterone and increases in testosterone in response to laboratory stress, area under the curve with respect to ground (AUCg) and area under the curve with respect to increase (AUCi) scores were calculated for each participant. AUC analyses are commonly utilized in study designs including repeated bio-sample measures and allow researchers to determine both overall biomarker secretion and acute changes in secretion (Pruessner et al., 2003). AUCg captures how much testosterone in total was secreted by participants across data collection timepoints, and, if one were to plot testosterone values of each sample across the study, is determined by calculating a sum of each trapezoidal space between testosterone values, the formula accounting for length of time between measures. AUCi scores were utilized to capture any increases in testosterone levels across administration of the TSST. This was accomplished by subtracting a pre-TSST baseline rectangle, again accounting for length of time between samples, from overall AUCg scores, thus providing a metric of how testosterone increased across TSST administration. AUCg and AUCi scores were both log-transformed for use in regression analyses. Log transformations of this variety are freuqnetly utilized by researchers utilizing AUC scores (Pruessner et al., 2003).

Measures of biopsychosocial predictors.

Biological predictors: age, sex, and pubertal development. Adolescents reported data on both their age and sex during the collection of demographic factors. Pubertal

development was assessed using the Pubertal Development Scale (PDS) (Petersen et al., 1988). Adolescents were asked to report on the status of various biological changes associated with puberty, including secondary sex characteristics of voice change and facial hair for males and breast development and menarche for females. Additionally, all adolescents reported on changes to skin and growth spurts. Adolescents reported on each item using a 1–4-point Likert scale, with 1 indicating "no development" and 4 indicating "completed development". For the female item capturing if menarche has occurred, scores of "no" were given a score of 1 and "yes" a score of 4. Both males and females responded to 5 items; the Cronbach's alpha was α = .80 and α = .73 for boys and girls, respectively.

Psychological predictors: behavioral and emotion regulation, affective reactivity. Parent-reported scores of both behavioral and emotional regulation were captured utilizing the Behavior Rating Inventory of Executive Function (BRIEF), a measure consisting of 86 items aimed at addressing overall executive abilities in adolescents and children aged 5-18 (Gioia et al., 2000). Parents were asked to report on their adolescent's behavior using a 3-point Likert scale, with a score of 1 corresponding to "Never," 2 to "Sometimes," and 3 to "Often." Example items include "Overreacts emotionally" and "Has problems waiting their turn." In all, the BRIEF contains 8 clinical sub-scales, made up of items aimed at addressing specific constructs within executive functioning. The current study utilized the behavioral and emotional regulation sub-scores of the BRIEF, specifically. Behavioral regulation scores consisted of the combined sub-scales of Inhibit and Self-Monitor, and had a Cronbach's Alpha of α = .86. Emotion regulation consisted of the combined sub-scales of Shift and Emotional Control and had a Cronbach's Alpha of and

showed strong internal reliability, α = .89. Previous studies have found the emotional and behavioral sub-scores to be distinct from one another (Egeland & Fallmyr, 2010).

Adolescent affective reactivity was captured utilizing the parent-report Affective Reactivity Index (ARI), a brief and widely-utilized tool for assessing proneness to anger and irritability in adolescent samples (Stringaris et al., 2012). The ARI consists of six items aimed at capturing dimensions of reactivity: outbursts of temper, touchiness, moodiness, and annoyance. Parents were prompted, "In the last 6 months and compared to others of the same age, how well does each of the following statements describe the behavior/feelings of your child?", and selected either "Not True", "Somewhat True", or "Certainly True" to each of the six items. Total scores were calculated as the sum of the six items, and the scale showed strong internal reliability, α = .84.

Social predictor: exposure to early life adversity. Adolescent reports of early life adversity were operationalized using The Early Trauma Inventory – Self Report (ETI-SR) (Bremner et al., 2007). The ETI consisted of 37 potential traumatic experiences that fit into one of five distinct subscales: General Trauma, Physical Abuse, Emotional Abuse, and Sexual Abuse. Adolescents were asked to indicate first if this experience has ever happened to them (0 indicating "no", 1 indicating "yes") and if it had, to report on the frequency using a scale ranging from 1 time (score of "1") to 5+ times (score of "5"). Chronicity scores were calculated by multiplying these two items ("Ever" and "Amount"), providing researchers with data that captures not only the occurrence, but also cumulative frequency, of exposure to early life adversities. Of note, in the current study, the sexual abuse sub-scale consisted

of a single item: “Have you ever experienced anything that you would consider sexual assault or sexual abuse?”.

Analytic plan

Goals of Aim 1 were explored by calculating mean scores of testosterone, in pg/mL, at each of the nine collection timepoints. T-tests of samples T4 and T5, which occur before and after TSST, respectively, were conducted to determine if there was a significant change in mean testosterone levels between these two time points. To address Aim 2, hierarchical regression models were utilized to explore the potential predictive capacities of seven biopsychosocial factors on both AUCg and AUCi. Both AUCg and AUCi scores were calculated following the parameters and formulae outlined in by Pruessner and colleagues (2003). Biological factors were explored first, and factors found to significantly predict AUCg or AUCi were included as covariates in models exploring psychological and social factors, to determine the effects of these factors above and beyond the influence of age or pubertal status. Further, given precedent in the field that accounts for well-known differences in levels of testosterone between boys and girls (Matchock et al., 2007), all psychological and social predictors were examined separately in boys and girls. All analyses were conducted using SPSS v.27.

RESULTS

General findings

AUCg scores (M_{AUCg} = 11642.85 pg/mL, SD = 7753.34) and AUCi scores (M_{AUCi} = 233.37 pg/mL, SD = 2490.75) were conducted for all participants. Descriptive statistics of all study variables can be found in Table 1. Regarding exposure to ELA, all participants confirmed experiencing at least some adversity exposure on the ETI. Specifically, items "Have you ever been spanked with a hand?" (54.6% of participants reporting yes), "Have you ever been slapped in the face?" (50.5% of participants reporting yes), and "Have you witnessed violence towards others?" (also 50.5% of participants reporting yes) were the most frequently endorsed items on the total ETI. Given recruitment protocol and overall study aims, these instances of exposure to ELA were expected.

Correlations between all study variables were conducted (Table 2). All biological predictors were found to be significantly correlated with AUCg (Age: $r(85) = .46$, $p < .001$ Pubertal Status: $r(84) = .26$, $p = .007$; Sex: $r(85) = .46$, $p < .001$). Overall exposure to ELA was correlated with AUCg, $r(85) = .36$, $p < .001$. AUCi, however, was not correlated as robustly with study predictors, and indeed, was only significantly correlated with sex, $r(85) = .23$, $p = .02$.

Acute testosterone reactivity to stress

To determine the impact of an acute laboratory stressor on testosterone levels, mean testosterone scores were calculated for each of the nine data collection time points (T1-T9). Analyses revealed that in the sample collected following the administration of a highly standardized TSST (T5), adolescent salivary testosterone levels rose (M_{T4} =

72.33pg/mL, M_{T5} = 82.70pg/mL). This effect was more pronounced in males, who displayed an average 15.84 pg/mL increase (between T4 and T5) in salivary testosterone following the administration of the TSST. Females displayed an average 5.02 pg/mL increase, a change that remains statistically significant at the p = .00 level. As displayed in Figure 1 adolescent testosterone levels decreased again between T5 and T6, reflecting a return to pre-TSST levels by the end of data collection (M_{T8} = 71.95, SD = 53.91).

Indeed, across the whole sample, 44 (48.8%) of the participants with complete testosterone data saw at least a 10% increase in testosterone levels between T4 and T5. Of these 44 participants, 19 (43.2%) were female. Among these participants, 32 (35.5% of total sample, 37.5% female) saw at least a 20% increase in mean testosterone levels following administration of the TSST. Figures 3 and 4 provide mean testosterone levels for samples T1-T9 in the overall sample, males, and females.

Biological, psychological, and social predictors of two indices of testosterone activity.

Biological predictors. A series of regression analyses were conducted to explore the predictive validity of sex, pubertal development, and age on both AUCg and AUCi. Sex significantly predicted AUCg [b = .50, t(82) = 5.99, p <.00], as did age [b= .41, t(82) = 4.17, p <.00], but pubertal status did not [b = .12, t(82) = 1.16, p =.25]. Age was the only biological factor that predicted AUCi [b = .30, t(82) = 2.38, p <.05]. Neither sex [b = .08, t(82) = .77, p =.44] nor pubertal status [b = -.13, t(82) = -.99, p =.33] significantly predicted AUCi.

Given these findings, age was included as a covariate in all remaining regression models. Further, in light of the previously established and presently observed significance

of sex in testosterone research, regression analyses of both social and psychological predictors were conducted separately in males and females. Male and female participants did not significantly differ in terms of age [$t(86) = .49$, $p = .63$] or pubertal status [$t(86) = 1.69$, $p = .09$].

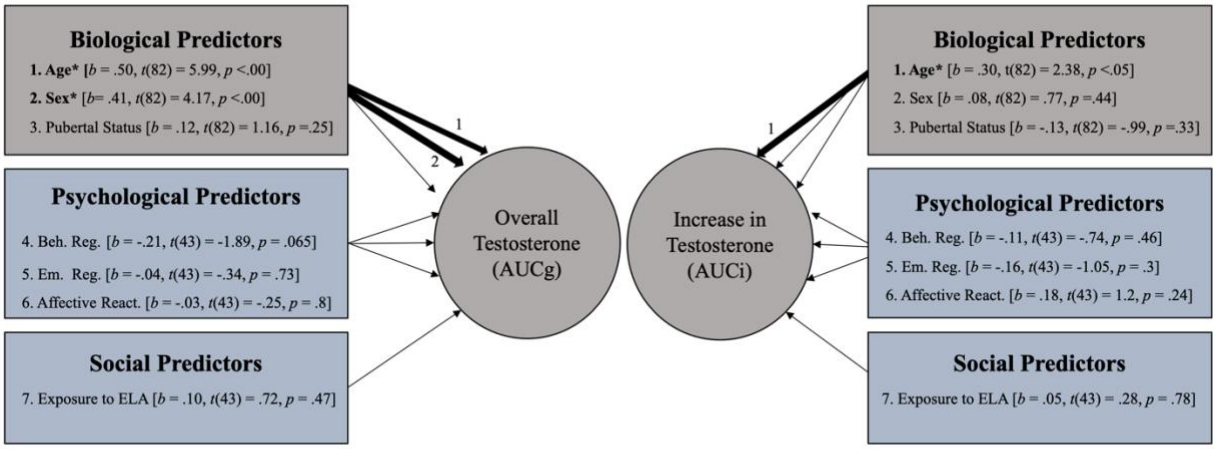
***Psychological predictors*.** Among male participants, only behavioral regulation approached significance when predicting AUCg scores [$b = -.21$, $t(43) = -1.89$, $p = .065$]. Meanwhile, emotional regulation [$b = -.04$, $t(43) = -.34$, $p = .73$] and affective reactivity [$b = -.03$, $t(43) = -.25$, $p = .8$] were not found to be significant predictors. Neither behavioral regulation [$b = -.11$, $t(43) = -.74$, $p = .46$], emotional regulation [$b = -.16$, $t(43) = -1.05$, $p = .3$], or affective reactivity [$b = .18$, $t(43) = 1.2$, $p = .24$] predicted AUCi in males. All models covaried for age.

Regressions examining psychological predictors of AUCg in females revealed that none of the variables were of significance—behavioral regulation [$b = .03$, $t(37) = .2$, $p = .84$], emotional regulation [$b = -.02$, $t(37) = -.11$, $p = .92$], and affective reactivity [$b = .05$, $t(38) = .31$, $p = .76$] did not predict AUCg. Interestingly, affective reactivity approached significance when predicting AUCi [$b = -.23$, $t(38) = -1.93$, $p = .06$], but behavioral regulation [$b = -.01$, $t(37) = -.62$, $p = .54$] and emotional regulation [$b = .05$, $t(38) = .31$, $p = .76$] did not, after covarying for age.

***Social predictors*.** To assess the influence of ELA on AUCg and AUCi indices, overall scores of ETI chronicity were explored as predictors of testosterone activity. For the males in our sample, AUCg was not predicted by overall ETI [$b = .10$, $t(43) = .72$, $p = .47$], which similarly did not predict AUCi [$b = .05$, $t(43) = .28$, $p = .78$]. Females displayed a similar

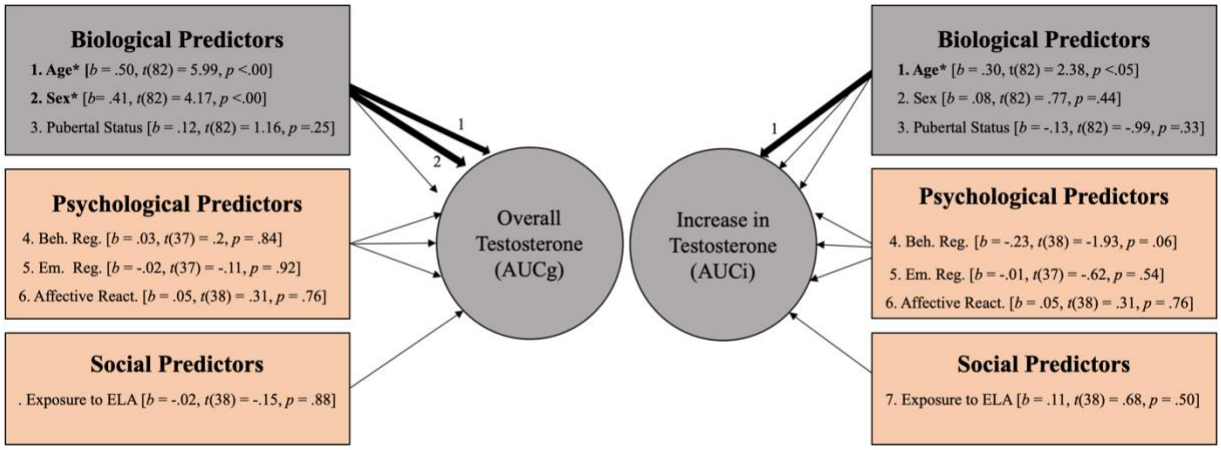
pattern of results, such that overall ETI did not predict AUCg [b = -.02, t(38) = -.15, p = .88] or AUCi [b = .11, t(38) = .68, p = .50]. Sub-scales of ETI influence were not explored, considering the lack of significant findings for overall ETI chronicity scores.

Figure 5. Biopsychosocial predictors of AUCg and AUCi in Males.



Note: * indicates $p < .05$. Results for psychological and social predictors are for male participants, results for biological predictors are for entire sample.

Figure 6. Biopsychosocial predictors of AUCg and AUCi in Females.



Note: * indicates $p < .05$. Results for psychological and social predictors are for female participants, results for biological predictors are for entire sample.

DISCUSSION

Despite the observed importance of testosterone in social and physical development, the exact role of testosterone in adolescent behavior is yet unknown (e.g. Rowe et al., 2004). Theoretical evidence suggests that considering testosterone in conjunction with stress contexts may provide the strongest framework for understanding pathways to problem behaviors (Archer, 2006), yet studies investigating whether testosterone mounts an acute stress response have failed to generate consensus (Deuter et al., 2021; Schoofs & Wolf, 2011). This may be due at least in part to inconsistencies in study design (i.e., the number and timing of bio-sample collection) that have made investigations into factors associated with acute testosterone reactivity challenging. The current study addressed this issue by analyzing mean salivary testosterone levels at nine points across the administration of a TSST, and determining if there was a significant difference in mean testosterone levels before and after TSST administration. Further, the current study examined patterns of two indices of testosterone activity (AUCg, which captures overall testosterone, and AUCi, which captures changes in testosterone following acute stress exposure) and explored the potential role of seven biopsychosocial predictors on scores for both indices.

To our knowledge, this is one of the only studies to explore whether adolescents exhibit an acute testosterone response to standardized psychosocial stressor (Bernhard et al., 2021; Johnson et al., 2020). Following the administration of the TSST, adolescent testosterone levels rose significantly, with the largest mean increase occurring between T4 (pre-TSST) and T5 (post-TSST). This effect was robust and notably present in both males and females. These findings contribute to studies supporting the existence of an acute

stress response in other samples (e.g., children [Drury et al., 2014]; healthy adult men and women [Lennartsson et al., 2012]), which may have important implications in future psychophysiological research. More directly, these findings imply that the observed mixed results concerning acute testosterone reactivity in response stress may be methodological in origin, rather than organic. To this end, findings from Aim 1 can be used to help pinpoint areas of consideration.

Methodological framework.

When comparing the methods of the current study to those of others that have found evidence both supporting and rejecting an acute testosterone response, a few areas emerge as potential sources of methodological clarification. However, it is crucial to note that research designs including biospecimen collection and analysis are both temporally and fiscally costly. As such, the following areas are organized in terms of general accessibility to researchers at large, with the ultimate goal to support feasible collection of acute testosterone reactivity to stress in other paradigms.

First, the number and timing of biospecimen collection may support the observation of an acute testosterone response. Previous studies that did not find an acute stress response included 2-3 samples in their study design (e.g., Schoofs & Wolf, 2011). In conjunction with previous research (e.g., Johnson et al., 2020; Lennartsson et al., 2012; Turan et al., 2015), findings from Aim 1 suggest a need for at least 4 data collection timepoints to capture an accurate testosterone response. As an extension, researchers may additionally consider the timing of specimen collection when aiming to capture an acute testosterone response. The current study was successful at capturing a pre-TSST baseline

within roughly an hour of arrival to the lab. Arrival to a research laboratory has been previously found to mount a stress response in most participants; this should be considered when collecting physiological data (Balodis et al., 2010). Study designs that collected baseline samples closer to lab arrival (for example, ~20 minutes after arrival, Schoofs & Wolf, 2011) were less successful at capturing change. Further, the current study collected samples immediately following TSST administration that captured a significant increase in testosterone, which is also found to be effective in other samples (e.g., Lennartsson et al., 2012). Our findings contribute to previous research suggesting the acute testosterone response is quite brief in nature—in our sample, decreases were observed within the 10-minute interval between T5 and T6.

In sum, researchers should aim to allow adequate time for baseline sample collection (at least 1 sample 45 minutes following lab arrival), then consider collecting multiple samples (~2) in shorter intervals (5-10 minutes) directly following the administration of a laboratory stressor, ideally followed by at least one sample 20-30 minutes after the stressor to allow for the capture of longer-term stress response patterns. Related to this issue, there is a well-established diurnal pattern of testosterone (a peak upon waking that decreases throughout the day) that may be important to researchers investigating testosterone reactivity, though this is decidedly standard practice and included in virtually all peer-reviewed studies (Rose et al., 1972).

The next consideration applies specifically to study designs utilizing the TSST to elicit a stress response. Unfortunately, we are limited in our capacity to make person-specific comparisons between examiners in other studies, yet it remains important to

discuss the steps of ensuring the effectiveness of a laboratory stressor. Examiners in the current study underwent rigorous training to perform the TSST consistently across examiners. Such training processes included practicing in front of the lab team, including superiors, and receiving detailed feedback on how to improve administration. Cues regarding facial expression, tone of voice, and delivery of script were given to all examiners, who were not cleared to be TSST judges until they were determined to be consistent with exact TSST instructions and with other examiners. Though we are unable to compare these training methods to those utilized in other studies, it is a potentially crucial consideration in such research designs.

Finally, the current study also had access to state-of-the-art salivary testosterone analysis. Wet lab protocols, along with biospecimen storage and transportation, may contribute to accuracy of findings. Notably, studies of a comparable design that utilized similar wet lab protocols also found results that indicated an acute testosterone response (ex: Drury et al., 2014), underscoring the importance of testing samples in two wells and generating a plate-specific standard curve. Concerning specimen storage and transportation, all data in the current study were collected within driving distance of the Institute for Interdisciplinary Salivary Bioscience Research center at University of California, Irvine, where storage and assaying processes took place. This helped ensure that all saliva samples were stored according to the highest of standards, with minimal travel time to reach the wet lab for assaying. Taken together, the accumulated effects of these methodological considerations may have contributed to the robust testosterone reaction captured in the current study.

Predictors of AUCg and AUCi.

As expected, Aim 2 findings indicated that biological predictors were most robust in their ability to predict testosterone activity. In particular, age and sex both uniquely predicted AUCg scores, while only age uniquely predicted AUCi scores. Notably, pubertal development was not a unique, significant predictor of either index. These findings likely reflect the fact that the most substantial rise in testosterone levels occurs at the very onset of puberty (Peper & Dahl, 2013), and as such, may precede many of the items included on the scale for measuring pubertal status. Indeed, only nine participants with available testosterone data were in prepubertal or early pubertal stages, and among female participants, 36 (80%) had begun menstruating, an event which signals late puberty.

Investigations into psychosocial predictors of testosterone activity, though lacking in statistical significance, nevertheless provide promising future directions. It is important to note that the purpose of Aim 2 was not to make causal inferences from these analyses, but rather to investigate the relationship between these indices of testosterone reactivity and previously established factors that either influence, or are influenced by, testosterone. Among boys in our sample, neither behavioral regulation, emotion regulation, affective reactivity, nor early life adversity significantly predicted AUCg or AUCi scores. Only behavioral regulation approached significance in predicting AUCg, after adjusting for age. A similar pattern was observed among females: predicting AUCi via affective reactivity was the only finding that approached statistical significance.

Though only approaching significance, the findings regarding unique predictors of AUCg and AUCi in males and females raise interesting implications for future research.

First, behavioral regulation scores in males approached significance in predicting AUCg, after controlling for the potential role of age. This would indicate that among adolescent males, inhibitory control and shifting ability (sub-scales of the BRIEF that make up behavioral regulation scores) may be linked with greater overall testosterone, such that overall testosterone and scores of behavioral regulation increases in tandem. Currently, however, these findings remain general, and a more in-depth analysis of these factors in a larger sample, or with more nuanced operationalizations of behavioral regulation, may be required to elaborate on the specific nature of these associations, and why they may not be present in females.

Among females in our sample, affective reactivity approached significance of negatively predicting AUCi scores, such that for every one unit increase in affective reactivity, there was a -.28 unit decrease in AUCi scores. Conceptually, this would indicate that as irritability and proneness to anger increased, female participants displayed smaller increases of testosterone in the face of an acute stressor. This finding appears be unsupported by current understandings of testosterone and aggressive behavior in the face of stressors, suggesting that greater reactivity may be related to greater aggression (Archer, 2006) and should therefore be explored in other samples to determine if such patterns remain. Indeed, these findings provide rudimentary evidence that predictors of testosterone reactivity may be distinct between adolescent males and females. Further investigation, including studies conducted with greater numbers of female participants, is needed to help define testosterone's role in adolescent female functioning.

Next steps and conclusions.

In addition to the direct methodological and conceptual contributions of this study, these findings may also add context to a growing body of literature that explores the connections between the hypothalamus-pituitary-adrenal (HPA) and HPG axes. Indeed, the findings of this study may encourage researchers investigating adolescent HPA-axis functioning to include measures of HPG-axis reactivity in future models. In particular, being able to include acute testosterone reactivity may enrich previous findings that have suggested that overall testosterone culminates in problem behavior more frequently in the context of low cortisol levels (Popma et al., 2007). Notably, links between problem behaviors and acute testosterone and cortisol levels in instances of psychosocial stress in adolescent populations remain unknown, despite being observed in adults (Romero-Martínez et al., 2013). The implications of Aim 1 support this as a possible avenue of future research.

Understanding the role of testosterone functioning during adolescence contributes to our understanding of problem behaviors in this population (Duke et al., 2014; van der Meij et al., 2010; Vogiatzi et al., 2021). With thoughtful methodological considerations, both male and female adolescents show testosterone reactivity to acute stress. However, many unknowns remain, and exploration of acute testosterone reactivity in other adolescent samples stands to further enrich this growing area of research.

Table 1. Descriptive statistics of all study variables.

	n	%		*Measure*	*M(SD)*	*Range (Min, Max)*
Gender			*Predictors*			
Male	44	53.6		Age	13.94(1.56)	11, 17
Female	40	46.4		Pubertal Status	8.19(2.34)	2, 12
*Racial and Ethnic Identiy**				Behavioral Regulation	17.8(4.23)	12, 33
White	67	72.2		Emotional Regulation	42.76(6.05)	16, 44
Black/African American	10	10.3		Affective Reactivity	2.38(2.41)	0, 10
Asian	17	17.5		Exposure to ELA	21.53(19.09)	0, 87
Native Hawaiian or Pacific Islander	2	2.1	*Testosterone Indices*			
American Indian or Alaska Native	3	3.1		Area Under Curve, Ground	1164.85(7753.34)	2681.75, 44008.5
Hispanic/Latinx	26	26.8		Area Under Curve, Increase	223.37(2490.75)	-6727.75, 5361

Note: * Some participants reported belonging to one or more racial/ethnic identities.

Table 2. Correlation coefficients of study variables in overall sample

	1	2	3	4	5	6	7	8	9
1. Age	1								
2. Sex	-0.03	1							
3. Pubertal Status	.58**	-0.15	1						
4. Behavioral Regulation	0.14	.24*	0.03	1					
5. Emotional Regulation	0.12	0.02	0.05	.54**	1				
6. Affective Reactivity	-0.11	0.01	0.04	.23*	.39*	1			
7. Exposure to ELA	.38**	0.13	0.08	0.19	0.04	0.03	1		
8. Overall Testosterone (AUCg)	.46**	.46**	.26*	0.05	0.03	-0.07	0.36	1	
9. Increase in Testosterone (AUCi)	.23*	0.09	0.03	-0.05	-0.06	-0.03	0.16	-0.46	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Figure 3. Mean testosterone across administration of TSST in total sample.

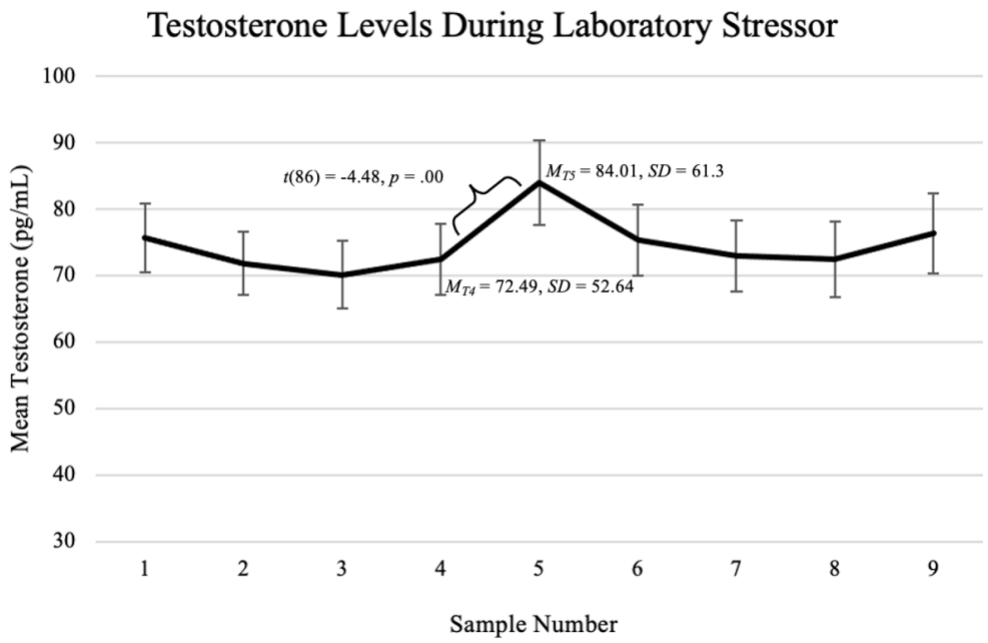
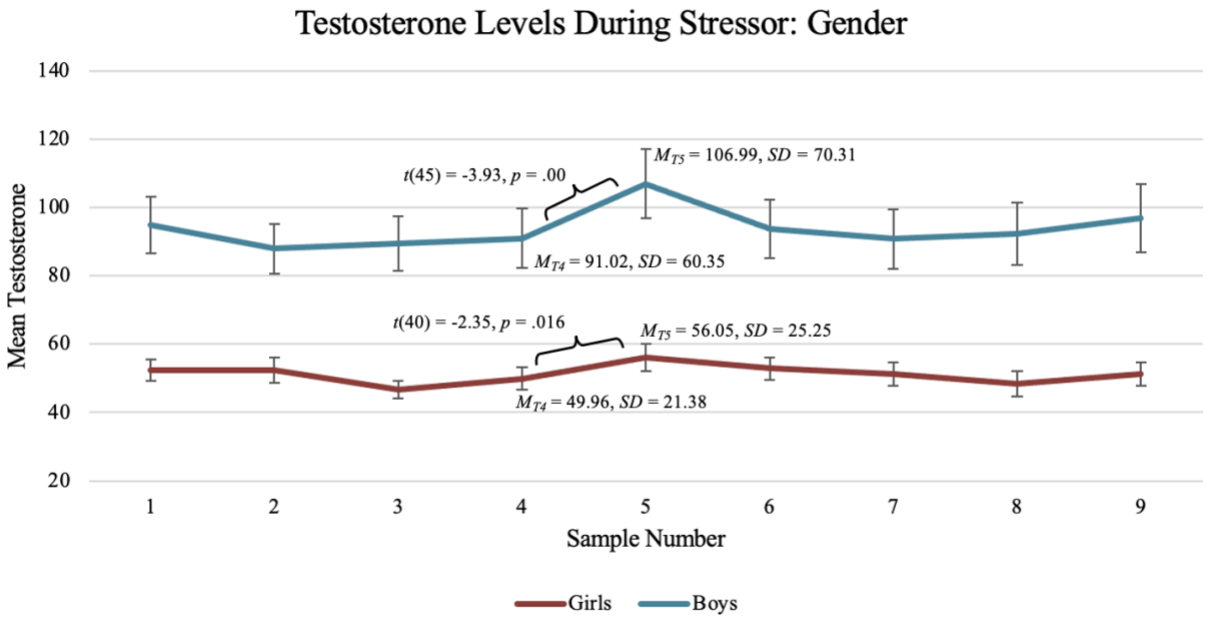


Figure 4. Mean testosterone across administration of TSST in boys and girls.



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