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INVESTIGATION OF THE PATHWAY FROM INTERPERSONAL STRESS TO INTERNALIZING PROBLEMS: POTENTIAL ROLES OF PUBERTAL TIMING AND HPA STRESS REACTIVITY

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ABSTRACT

Internalizing problems, rates of which increase during adolescence, is often linked to previous stressful experiences. Such stressful experiences that precede internalizing psychopathology are often of an interpersonal nature. Additionally, females are at higher risk for internalizing psychopathology during this developmental period. Sex differences in associations between interpersonal stress and internalizing problems during this time may be driven by pubertal and physiological stress reactivity processes. An investigation into whether sex differences exist in the interrelations between interpersonal stress, internalizing psychopathology, pubertal timing, and HPA stress reactivity (measured by cortisol reactivity to a social stress task) was conducted with a sample of 152 fourth and fifth graders in the northeastern United States. Interpersonal stress was significantly associated with internalizing problems in the full sample, but pubertal timing did not significantly mediate this relation in either females or males. Additionally, cortisol stress reactivity did not moderate the effect of interpersonal stress on pubertal timing. Alternative models were explored. Though pubertal timing and HPA stress reactivity were not found to influence the association between interpersonal stress and internalizing problems in this investigation, further examination of how sex differences in internalizing psychopathology emerge during adolescence is needed.

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INTRODUCTION

Rates of internalizing psychopathology increase dramatically during adolescence. This is especially true for females whose prevalence rates of depression, for example, skyrocket to 21% by mid-adolescence and remain double that of males into adulthood (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Thapar, Collishaw, Pine, & Thapar, 2012). It is not entirely clear why these mental health problems onset drastically for females during adolescence, though leading theories point to stress and pubertal processes. Better understanding of the mechanisms that transmit heightened risk for internalizing problems for adolescent females will help inform prevention and treatment interventions of internalizing disorders for females.

Psychosocial and psychobiological theories of stress such as those posed by Belsky, Steinberg, and Draper (1991), Trickett and Putnam (1993), and Del Giudice, Ellis, and Shirtcliff (2011) suggest that pubertal timing, hypothalamic-pituitary-adrenal (HPA) axis reactivity, and differential exposure to stressful life events during childhood may contribute to both elevations of internalizing problems generally, and for females, in particular. Interpersonal stressors in particular may play a unique role in the etiology of internalizing disorders (Cicchetti & Toth, 1998; Hammen, 1992). These distinct and complex constructs as well as how they may be related to each other will be further discussed in following sections.

The HPA axis is thought to contribute to internalizing psychopathology via its role in the body's stress response. When confronted with a stressor, the body normatively undergoes allostasis, a process in which homeostasis is maintained via various physiological responses. Through one such process, referred to as the HPA axis cascade, cortisol is released into the body via a chain of events occurring at the hypothalamus, the pituitary gland, and the adrenal gland. After serving as regulator for cardiovascular, metabolic, immune, and behavioral processes necessary to return the body to homeostasis, cortisol then shuts down the HPA axis stress response via a negative feedback loop (Smith & Vale, 2006). This is an adaptive process, and dysregulation of the HPA axis, either through high or low responsivity, can lead to psychopathology. Repeated and/or prolonged activation of the HPA axis, oftentimes via chronic exposure to stress, can lead to allostatic load, a "wear and tear" on the body, and changes to brain structures (i.e., hippocampus, amygdala) implicated in internalizing disorders (McEwen, 2003).

Sex differences are evident during the adolescent period in reported exposure to stressful life events, especially those of an interpersonal nature (Hankin, Mermelstein, & Roesch, 2007; Shih, Eberhart, Hammen, & Brennan, 2006), pubertal timing (Wolf & Long, 2016), salivary cortisol stress reactivity (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001), and in the inter-relations among these constructs (Natsuaki et al., 2009; Peckins, Dockray, Eckenrode, Heaton, & Susman, 2012; Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). How these constructs each contribute to internalizing problems and the extent to which sex differences in internalizing problems therein emerge during early adolescence, have yet to be examined simultaneously in one study. Therefore, this study builds on previous theories of both puberty and HPA pathways of stress's effects on mental health outcomes, to test a model in which pubertal timing mediates and HPA stress reactivity moderates the association between interpersonal stress and internalizing problems, specifically for females. The nature of the hypothesized mediation and moderation effects will be discussed in relation to previous findings.

Stress links to negative outcomes

Stressful experiences that occur in the first decade of life may be one mechanism that sets the stage for the development of internalizing problems in adolescence and onward, supporting the need to better understand mechanisms occurring during the turning point of early adolescence. Chronic stress, especially when first occurring during childhood, is consistently implicated in the etiology of mental health problems (Juster et al., 2010) throughout the lifespan. For example, the significant role played by childhood stress on the development of internalizing problems was demonstrated in McLaughlin, Conron, Koenen, and Gilman's (2010) examination of the stress sensitization hypothesis. Their findings showed that individuals who experienced at least three childhood stressful conditions (e.g., neglect, family conflict, physical and sexual abuse) were at higher risk for internalizing psychopathology such as depression and anxiety following the experience of an adulthood stressful event (McLaughlin et al., 2010). This supports the theory that experiences of childhood stress increase risk for having internalizing psychopathology within twelve months of exposure to stressors during adulthood. This study and a cadre of related empirical evidence suggest that interventions that aim to prevent and/or decrease the risk of developing internalizing problems should be targeted during adolescence.

Research and interventions should focus on early adolescence in particular, because it is the turning point during which biological, cognitive, and social changes are most salient and dramatic relative to development occurring during late childhood (Hamburg & Takanishi, 1989). Animal models (Bingham et al., 2011) serve as precedence to the notion that stress experienced during early adolescence, particularly that of a social nature, are especially impactful on physical and behavioral development. Additionally, sex differences in prevalence of internalizing problems first become evident during early adolescence (Hankin et al., 1998), making it a critical developmental period to study when trying to better understand mechanisms that underlie the etiology of internalizing psychopathology.

Studying exposure to interpersonal stress specifically may also help explain why internalizing psychopathology can develop during adolescence. Flynn and Rudolph (2011) found that interpersonal stress predicted depression while non-interpersonal stress did not in a study of female and male adolescents. Effects of interpersonal stress on depression have also been found in adults. In Sheets and Craighead's (2014) examination of predictors of major depressive disorder (MDD) recurrence in young adults, chronic interpersonal stress predicted greater risk of recurrence of MDD while chronic non-interpersonal stress did not have an effect on MDD recurrence. Developmental interpersonal stress theories of depression suggest that such experiences (e.g., interpersonal conflict, dysfunctional relationships) during childhood make individuals more vulnerable to developing depression through "maladaptive" developmental changes such as being more likely to make negative attributions to stimuli and developing a diminished sense of self-worth (e.g., Cicchetti & Toth, 1998; Hammen, 1992). Additionally, the nature of early adolescence as a point during which significant social changes start to occur make it more likely that interpersonal problems arise during this time and go on to contribute to internalizing problems.

Pubertal timing's pivotal role in negative outcomes

Effectiveness of interventions targeting internalizing problems may depend on the specific timing of administration, particularly with regard to the developmental period (e.g., mid-childhood, early adolescence, mid-adolescence). The peripubertal period in particular, appears to be a critical time during which the saliency of stress increases alongside the maturation of

multiple avenues for stress regulation or amplification (Romeo, 2010). The beginning of pubertal development may therefore serve as a natural touchpoint for interventions that could contribute to the development and potential recalibration of biological processes that can protect from childhood stress effects. In this section, evidence will be provided for why the peripubertal period is a sensitive period for changes in behavioral outcome trajectories due to concurrent development of the HPA axis. Additionally, the timing of onset of puberty relative to same-age, same-sex peers, henceforth referred to as pubertal timing, and how it is linked to outcomes is discussed.

The HPA axis is a neuroendocrine system involved in stress regulation and amplification that is susceptible to biological and environmental changes during puberty. The HPA axis is notably sensitive to stress exposure during early adolescence (Herman et al., 2016; Romeo, 2010), which suggests that both positive and negative changes to HPA functioning could be made during this developmental period. Additionally, HPA axis functioning in response to stress is consistently linked to dysregulation (either hyper- or hyporeactivity) and thought to be involved in the development and maintenance of depression (Burke, Davis, Otte, & Mohr, 2005; Colich, Kircanski, Foland-Ross, & Gotlib, 2015; Lopez-Duran et al., 2015) and anxiety (Yoon & Joormann, 2012). Gunnar and colleagues (2019) recently found that individuals who experienced significant early stress along with subsequent corrective nurturing experiences displayed lower HPA stress responses during the early stages of puberty but went on to display more normative HPA stress reactivity later on when they reached the later stages of puberty. These findings suggest that the pubertal period is a sensitive window during which physiological systems can recalibrate (Gunnar, DePasquale, Reid, & Donzella, 2019). If this is indeed true, it allows for interventions to capitalize on this period in order to potentially reverse effects of childhood stress.

But it is not enough to select a general age when puberty is expected to start for interventions that seek to leverage the peripubertal period as a sensitive period. It is imperative to note that timing of pubertal onset is not consistent across all individuals. For example, experiences such as undergoing extreme amounts of exercise (Warren, 1980) are associated with late pubertal timing while exposure to physical and sexual abuse (Boynton-Jarrett et al., 2013; Noll et al., 2017) are linked to early pubertal timing. Knowledge of what influences individuals' pubertal timing and in turn how pubertal timing can influence the relationship between stress and mental health outcomes is key for understanding the mechanism through which stress and internalizing problems are connected.

A major theory of how differences in pubertal timing occur comes from Belsky and colleagues (1991), who tied in influences from modern evolutionary theory, behavioral ecology, and sociobiology to theorize that pubertal timing is influenced by early experiences that signal the likelihood of reproductive success of individuals in their respective environments. This theory contends that early stressful experiences, especially experiences of parental absence, harsh parenting, and stressors that occur in the family and home setting, signal to the individual to expect similar unstable relationships and unpredictable and/or limited resources in the future. This expectation then spurs the individual to enter puberty earlier in order to biologically prepare for reproduction earlier and work to ensure reproductive success in the face of an expected unwelcome environment. To date, evidence for this psychosocial acceleration theory include findings that early pubertal timing has been associated with a wide range of childhood stressors, including family conflict (Saxbe & Repetti, 2009) and harsh parenting (Belsky, Steinberg, Houts, & Halpern-Felsher, 2010).

Trickett and Putnam (1993) put forth a similar model regarding specific effects of child sexual abuse on mental health outcomes in females. In this model, exposure to child sexual abuse is thought to impact pubertal development via earlier pubertal onset, which then is thought to increases risk for depression and anxiety. Similar to evidence for the psychosocial acceleration theory, evidence for Trickett and Putnam's model supports the relation between stress and earlier pubertal timing (e.g., sexual abuse associated with early pubertal timing in females (Mendle, Leve, Van Ryzin, & Natsuaki, 2014; Negriff, Blankson, & Trickett, 2015)).

Though early pubertal timing may be an adaptive reproductive "strategy" in the face of childhood stress, it is also linked with increased rates of negative behavioral outcomes as well as physical health risks. Early pubertal timing in females has previously been linked to internalizing problems (Hamilton, Hamlat, Stange, Abramson, & Alloy, 2014; Kim & Smith, 1998; Mendle, Turkheimer, & Emery 2007). Behaviorally, earlier pubertal timing as measured by earlier age of menarche, predicted greater sexual risk taking in females (Belsky et al., 2010). Physically, earlier pubertal timing, also measured by earlier age of menarche, predicted increased cardiovascular risk in women participating in a study of reproductive aging (Bleil et al., 2013). Bleil and colleagues (2013) also found evidence that this relationship was preceded by higher rates of childhood stressors, further supporting the theory that the link between childhood stress and negative health outcomes may be mediated by pubertal timing. Other evidence for theories connecting stress to negative outcomes via pubertal timing includes the finding that pubertal timing partially mediated the relationship between increased childhood stress and greater sexual risk taking in females, suggesting that childhood stress's causal effect on risky sexual behaviors is partly due to earlier pubertal timing (James, Ellis, Schlomer, & Garber, 2012). Thus, well-established links between childhood stress and negative outcomes may be mediated by pubertal timing (Joos, Wodzinski, Wadsworth, & Dorn, 2018). This study will focus on internalizing problems as the negative outcome that may be caused by interpersonal stress due to earlier pubertal timing.

HPA axis functioning in relation to stress, mental health, and pubertal timing

Though some evidence has supported the mediational nature of pubertal timing on the relationship between childhood stress and psychological problems, it is unclear through which biological mechanism these associations may occur. A strong candidate is the stress response of the HPA axis. Previous stressful experiences, particularly childhood stress, are often linked to HPA axis functioning and are thought to have influences on HPA regulation in response to threats and challenges. For example, in a study examining timing of stressful experiences and HPA stress reactivity, Bosch et al. (2012) found that stress (i.e., hospitalization, parental divorce, death of family member, out-of-home placement, parental addiction, parental mental health problems) experienced during ages 6-11 was linked to hyperreactivity of the HPA axis at age 16, as measured with stress cortisol levels, to a social stress test in males and females. Among the types of stress that are connected to HPA functioning in both males and females are poverty (Blair et al., 2013; Evans & Kim, 2007), maltreatment (Harkness, Stewart, & Wynne-Edwards, 2011; MacMillan et al., 2009), trauma (Klaassens et al., 2009), exposure to violence (Aiyer, Heinze, Miller, Stoddard, & Zimmerman, 2014; Peckins et al., 2020), and physical abuse (Carpenter, Shattuck, Tyrka, Geracioti, & Price, 2011). Some forms of stress were associated with hyporeactivity (i.e., poverty, trauma, exposure to violence, physical abuse) while maltreatment was associated with both hyper- and hyporeactivity.

Furthermore, both hyper- and hyporeactivity of the HPA axis to stress are associated with psychological problems. Ouellet-Morin et al. (2011), in a study of maltreated and/or bullied adolescents, found that experiences of victimization were related to cortisol hyporeactivity, which in turn predicted increased reports of social and behavioral problems on the Child Behavior Checklist (CBCL). As for internalizing psychopathology in particular, cortisol hyperreactivity in 6th, 7th, and 8th graders was related to increases in their internalizing symptoms across the following two years (Koss, Cummings, Davies, & Cicchetti, 2017). A meta-analysis of relations between cortisol stress reactivity and psychopathology identified internalizing disorders as mental health disorders associated with cortisol hyporeactivity in adults (Zorn et al., 2017). This meta-analysis also found support for sex differences in driving these relations. Women who have cortisol hyporeactivity are more likely to have an internalizing disorder while men who have cortisol hyperreactivity are at greater risk for depression and anxiety (Zorn et al., 2017).

Because both HPA regulation and pubertal timing are associated with stress and mental health outcomes, it is possible that they interact with each other and together serve as a mechanism through which childhood stress is strongly associated with psychological functioning. There is existing evidence that supports this proposition – Colich et al. (2015) assessed cortisol stress reactivity of adolescent females who were at different levels of pubertal development. They found that pubertal development interacted with cortisol stress reactivity to predict the onset of MDD, such that cortisol hyporeactivity predicted MDD onset for females who were assessed when they were at the early pubertal stages while cortisol hyperreactivity predicted MDD onset for females who were assessed when they were at the later pubertal stages. Additionally, cortisol stress reactivity naturally increases with pubertal stage, which might contribute to the increased risk for psychopathology that is often observed during adolescence (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Stroud et al., 2009; Sumter et al., 2010). Exposure to stressors, especially those of an interpersonal nature, that put individuals at higher risk for psychopathology also increase and become more salient during this developmental period (Hankin et al., 2007; Shih et al., 2006). For individuals who experience pubertal onset earlier than their peers and encounter high levels of interpersonal stress, it may be that the additional presence of high HPA stress reactivity increases their risk for developing psychological problems via a moderation effect.

There is existing evidence for HPA stress reactivity as a moderator on the association between stress and mental health outcomes, but such investigations did not consider pubertal timing as an additionally contributing factor. Hagan and colleagues (2014) examined the relation between child maltreatment as a specific type of stress and psychopathology outcomes in young adults. They found that this relation was moderated by HPA stress reactivity, such that child maltreatment was related to internalizing symptoms for individuals who exhibited higher cortisol reactivity to a conflict role-play task (Hagan, Roubinov, Mistler, & Luecken, 2014). HPA stress reactivity was also found to have a moderating effect on the association between stressful family events and psychological problems in adolescents (Steeger, Cook, & Connell, 2017). In this study, stressful family events and both internalizing and externalizing problems were positively associated for adolescents who had cortisol hyperreactivity to a conflict interaction task (Steeger et al., 2017). Though there is promising evidence that HPA stress reactivity moderates associations between stress and mental health, studies testing pubertal timing as a driving force behind these relations as posited by models identifying pubertal timing as a mediator, have not yet been conducted.

Sex differences

Given that there are sex differences in internalizing psychopathology that emerge in early- to mid-adolescence, it is important to better understand how the presence of sex differences in stressful experiences, pubertal timing, and HPA stress reactivity may contribute to sex-differentiated mental health problems. Generally, there are increases in prevalence of mood disorders during adolescence (Merikangas et al., 2010). Within the adolescent population, females are up to twice as likely to be diagnosed with an internalizing disorder compared to their male counterparts (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Hankin et al., 1998; Rutter, Caspi, & Moffitt, 2003).

Sex differences in interpersonal stressful experiences may heavily contribute to the female-driven increase in internalizing psychopathology seen during adolescence. Notable evidence for this mechanism comes from Hankin and colleagues' (2007) longitudinal examination of how stress experienced during adolescence in a group of 8th and 10th grade students influenced level of depressive symptoms across 12 months in females versus males. It was found that females generally reported both increased amounts of stress exposure, particularly in interpersonal contexts, and higher levels of depressive symptoms than males (Hankin et al., 2007). Most importantly, elevated stress exposure partially mediated the concurrent association between sex and depressive symptoms at each of the three data collection waves of the study (Hankin et al., 2007). Shih and colleagues (2006) also found evidence that interpersonal stress explained sex differences in adolescent depression. In a cross-sectional study of 15-year-old adolescents, females were more likely to have been exposed to interpersonal stress than males (Shih et al., 2006). Additionally, relatively higher rates of depressive disorders in these females were explained by their higher risk of exposure to interpersonal stress (Shih et al., 2006). Thus, higher rates of internalizing psychopathology in females may be explained in part by increased risk for exposure to interpersonal stressors once they start adolescence.

The role of pubertal timing in influencing the association between childhood stress and internalizing problems may also be sex-dependent. Pubertal timing may be a mediator of the relationship between stress and behavioral functioning, and James et al. (2012) found that pubertal timing's mediating influence on the stress-behavioral outcomes relationship was significant for females but not males. Stress is thought to be more salient for females than males in regard to its effect on pubertal development. Theories as to why this may be find their origins in Draper and Harpending's (1982) and Belsky et al.'s (1991) theories that postulate that childhood stress plays a role in pubertal maturation and, subsequently, reproductive strategy for females who aim to maximize their reproductive success in an evolutionary perspective. The Adaptive Calibration Model (ACM; Del Giudice et al., 2011), a more contemporary theory regarding physiological mechanisms connecting stress and psychopathology, focuses on how differences in pubertal development can be the result of adaptive evolutionary strategies in response to stress. Sex differences in adaptive pubertal development could then be the product of sex-differentiated reproductive strategies in which it is costlier for females than males to reproduce, particularly in contexts of chronic stress (Del Giudice et al., 2011).

Because HPA regulation is thought to also play an important role in the development of psychological problems, sex differences in HPA stress reactivity must also be considered. The ACM proposes sex-differentiated stress responses in the context of high levels of stress so that females display high responsivity while males display low responsivity in high-risk (e.g., severe chronic stress, trauma) environments (Del Giudice et al., 2011). Thus far, researchers have observed cortisol reactivity sex differences in adult and adolescent humans to an acute social

stressor (e.g., Kirschbaum, Wüst, & Hellhammer, 1992; Mazurka, Wynne-Edwards, & Harkness, 2018). Additionally, some sex differences in stress reactivity and neural maturation emerge during early adolescence. Males start to show greater cortisol reactivity than females (Ordaz & Luna, 2012) while females experience smaller age-related decreases in gray matter volume and smaller increases in white matter than males starting in early adolescence (De Bellis et al., 2001). Though these existing sex differences in stress responsivity were not observed in the context of high-risk environments suggested by the ACM, these findings are nevertheless suggestive that sex differences in internalizing symptoms during adolescence may manifest through sex-dependent mechanisms such as the development of HPA-axis regulation.

There has already been some work conducted in order to better understand how both pubertal timing and HPA stress reactivity contribute to internalizing problems in ways that are sex-specific. Natsuaki et al. (2009) found, for example, that early pubertal timing predicted increased internalizing symptoms and that this relationship was partially explained by increased salivary cortisol stress reactivity in females but not in males. In sum, there is evidence that early life stress is associated with elevated risk for the development of internalizing psychopathology and that this risk may be conveyed by stress-induced calibrations of the HPA-axis and pubertal timing. Furthermore, females' elevated levels of interpersonal stress and stress-linked pubertal differences, suggest that HPA and puberty effects stemming from childhood chronic interpersonal stress may underlie the sex differentiation in rates of internalizing problems that emerges during adolescence. Therefore, this study tests the extent to which pubertal timing mediates the effects of childhood interpersonal stressors on internalizing problems and whether HPA stress reactivity moderates this mediation effect.

Current study

The purpose of the current study was to examine potential sex differences in the inter-relations between childhood interpersonal stress, internalizing psychopathology, pubertal timing, and HPA stress reactivity in order to better understand why females become more at risk for internalizing disorders starting in adolescence. In a community sample of female and male early adolescents in fourth and fifth grade, childhood interpersonal stressful life events, internalizing problems, and pubertal development were assessed via parent-report measures while adolescents provided saliva samples before and after completion of a laboratory social stress task for HPA stress reactivity in a cross-sectional study.

A first-stage moderated mediation model in which the indirect effect of childhood interpersonal stressful life events on internalizing problems via earlier pubertal timing is predicted to depend on different levels of cortisol stress reactivity (Figure 1) was tested in the whole sample, then separately in females and males to determine potential specifically sex-driven relations. A moderated mediation states that the relation between two variables (interpersonal stress and internalizing problems) is mediated (i.e., caused) by a third variable (pubertal timing) and that this mediation is moderated (i.e., influenced) by a fourth variable (cortisol stress reactivity). This hypothesized model was formed based on theories regarding interpersonal stress as a unique predictor for the development of internalizing disorders (e.g., Cicchetti & Toth, 1998; Hammen, 1992), theories regarding pubertal timing as a mediator between childhood stress and psychosocial outcomes (Belsky et al., 1991; Trickett & Putnam, 1993), and theory regarding physiological responsivity to stress as a sex-specific response (Del Giudice et al., 2011) that puts females at higher risk for internalizing psychopathology.

Hypotheses

Hypothesis 1: As suggested in the study by James and colleagues (2012), in which pubertal timing partially mediated the effects of childhood stress on behavioral outcomes (e.g., risky behavior), we predicted that pubertal timing will mediate a relationship between experiences of interpersonal stress in the past 12 months and internalizing problems, such that higher number of reported past stress events will predict increased internalizing problems via earlier pubertal timing.

Hypothesis 2: Based on previous findings that HPA stress reactivity can moderate the relation between stressful experiences and internalizing symptoms (Hagan et al., 2014; Steeger et al., 2017), we predicted that cortisol reactivity will moderate the mediation effect of pubertal timing on the relation between past experiences of interpersonal stressful events and pubertal timing, such that the mediation effect is stronger when cortisol reactivity is higher (i.e., cortisol hyperreactivity).

Hypothesis 3: Hankin et al. (2007) and Shih et al. (2006) provided compelling evidence that interpersonal stress exposure partially explains why internalizing problems such as depressive symptoms are much more prevalent in females starting in adolescence. Associations between childhood stress and earlier pubertal timing are also found to be significant for females but not males (James et al., 2012). Additionally, females' increased stress cortisol reactivity was implicated in Natsuaki and colleagues' (2009) finding that earlier pubertal development was associated with internalizing problems. As such, we predicted that the overall moderated mediation model described by Hypotheses 1 and 2 will differ by sex, such that all model effects will be significant for females but not for males.

METHODS

Participants

Data were collected from 152 fourth and fifth grade children recruited from schools in the northeastern United States for a cross-sectional study on stress and coping during early adolescence. One hundred twenty-six participants had complete data for the current study's variables of interest and were thus included in analyses (M_{age} = 10.54 years, *SD* = 0.76, range = 7.13-12.06, 50% female). The remaining 26 participants were excluded in analyses due to missing data for at least one of the following measures: recent stressors, pubertal status, salivary cortisol, and internalizing problems. The 126 participants included in this study did not differ significantly from the 26 excluded participants in any of the variables of interest (*p*s > .05) except for pubertal status (operationalized in the "Measures" section), $t(143) = -2.16$, $p = .03$. The excluded participants ($M = 1.42$) had lower pubertal status than the included participants ($M = 1.70$); however, excluded ($M = -0.20$) versus included ($M = 0.002$) participants did not differ on the derived pubertal timing measure that was ultimately used in analyses, $t(128) = -0.89$, $p = .38$. Additionally, included and excluded participants did not differ on pubertal status within the subset of males, $t(75) = -0.95$, $p = .34$, or females, $t(66) = -1.24$, $p = .22$. Subsequent analyses reported are representative of this subset of participants.

Child participants identified as Native American/Alaskan (1.6%), Asian (1.6%), White (92%), or Other (4.8%). About 97% of the child participants also identified as non-Hispanic or Latino while 3.2% identified as Hispanic or Latino.

Each child had one parent (88.8% females) who participated in the study with them. Parents identified as Asian (2.4%) or White (97.6%). About 99% of the parents also identified as non-Hispanic or Latino while 0.8% identified as Hispanic or Latino.

A wide range of SES is represented in the sample as assessed by parent report of annual household income (M = \$109,610, *SD* = \$163,087, range = \$13,639-\$1,300,000, *Mdn* = \$74,500).

Procedures

Sessions were scheduled with the child participants and their parents between 3:00 and 5:30 pm. Participants were instructed to refrain from eating or brushing their teeth within an hour of the start of their sessions – this was done to avoid effects of food intake on salivary cortisol levels (Gibson et al., 1999). Parents and children completed questionnaires throughout the session.

Figure 2 depicts the study's assessment timeline. Upon arrival at the laboratory, an experimenter first administered questionnaires to the child participants. After 40 minutes of completing questionnaires, the child participants underwent the Trier Social Stress Test for Children (TSST-C), where they were instructed to tell a story and perform mental arithmetic in front of a judge (Kirschbaum, Pirke, & Hellhammer, 1993). Experimenters video recorded the child participants throughout the TSST-C. Children were given five minutes to prepare for a speech based on a prompt that was given to them. Then, the participants had five minutes to deliver this speech to two neutral-faced confederate judges. Following the speech task, the judges asked the participants to complete a mental subtraction task for five minutes.

Due to the larger study's focus on effects of coping, the children completed one of two coping conditions after completion of the TSST-C. The participants were randomly assigned to either the avoidance coping condition, in which they were placed in a room without engaging

stimuli and asked to not think about their performance on the TSST-C, or to the distraction coping condition, in which they were placed in a room with toys, art supplies, and musical instruments and were given permission by the experimenter to play with any of the materials. The children spent 10 minutes in the coping condition. Because participants underwent different coping conditions during stress recovery, current analyses focused on stress reactivity where all participants received the same stressor condition. After this 10-minute period, the experimenter interviewed the children and asked scripted questions about the coping strategies that the children used during the coping condition. The children then participated in a guided progressive muscle relaxation exercise for 10 minutes. After the progressive muscle relaxation task, children completed the rest of their questionnaires.

Salivary cortisol, a reliable measure of HPA-axis activity, was measured seven times throughout the session (Hellhammer, Wüst, & Kudielka, 2009). The timing of saliva samples is shown in Figure 2. The first saliva sample was taken when the children first arrived for their sessions. The children provided the second saliva sample 40 minutes after the first sample and right before beginning the TSST-C. The third saliva sample was administered 15 minutes after the second sample and right after completion of the TSST-C. The fourth saliva sample was taken 10 minutes after the third sample and right after completion of the coping condition. The experimenter collected the fifth saliva sample from the children 10 minutes after the fourth sample and right after the coping interview. The sixth saliva sample was administered 10 minutes after the fifth sample and right after the guided progressive muscle relaxation exercise. The seventh and final saliva sample was collected 10 minutes after the sixth saliva sample.

Measures

Interpersonal stressors. Parents reported childhood stressors that their children had experienced on the Child and Adolescent Survey of Experiences: Parent Version (CASE-P; Allen, Rapee, & Sandberg, 2012). The CASE-P is a checklist of items that are meant to capture acute life stressors that have occurred within the past 12 months. The CASE-P consists of 38 items that assess for life events ranging from moves, separation from family or other loved ones, illness or injury, family conflict, and difficulties at school. In this study, only the 31 CASE-P items that illustrated negative life events were administered to parents of participants. If an event was endorsed, the parent was asked to rate the impact of the event on a Likert scale of 1 to 3 (1 = a little bad, 2 = quite bad, 3 = really bad). The CASE-P has good validity, interrater reliability, and test-retest reliability (Allen et al., 2012). Internal consistency, α, was 0.62 for the full sample, 0.52 for males, and 0.70 for females. Youth were reported to have experienced relatively fewer stressors compared to higher-risk samples referred to in the ACM (M = 4.79, SD = 2.82, range = 0-15, Mdn = 4).

This study focused on the interpersonal stress items of the CASE-P (i.e., “My child was teased or bullied”, “My child had a big argument with someone in our family”, “My child broke up with a boyfriend or girlfriend”, “My child had a big argument with someone special to him/her (who is not family)”, and “My child was in a fight (not with people in our family)”). There was a total of five events on the CASE-P that were interpersonal in nature. The number of endorsed negative interpersonal life events was summed to create a total score of interpersonal events. Internal consistency, α, for the subset of interpersonal stress items was 0.48 for the full sample, 0.35 for males, 0.59 for females.

Pubertal status and timing. Parents completed the parent-report Pubertal Developmental Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988). This measure is valuable in

determining subjective reports of pubertal status. Parents were asked to rate their child's current pubertal status on height, body hair growth, skin changes, and sex-specific items (for females: breast growth, menarche; for males: voice deepening, facial hair growth). Internal consistency, α, was 0.80 for female pubertal status and 0.28 for male pubertal status. The five items were averaged to obtain the pubertal status measure separately by sex. The PDS has good validity and acceptable reliability (Carskadon & Acebo, 1993), though internal consistency for males in the current sample was weak. This may be because most of the parents reporting on their children's pubertal status were female; mothers may be more likely to be privy to the different physical characteristics of puberty in their daughters than in their sons (Brooks-Gunn, Warren, Rosso, & Gargiulo, 1987; Dorn, Susman, Nottelmann, Inoff-Germain, & Chrousos, 1990).

Pubertal timing was calculated by regressing pubertal status on chronological age and obtaining the residuals separately by sex (Susman et al., 2007). Though there are other ways to measure pubertal timing (Dorn & Biro, 2011), using the regression method was the best way to utilize the one-time assessment of pubertal status to determine timing in a sample that was mostly at the earlier stages of puberty. The residual score reflected the parent perception of each child participant's pubertal development in relation to the regression line's expected value for the child's age. A negative residual score indicated that the individual had later pubertal timing than expected for their age while a positive residual score indicated that the individual had earlier pubertal timing than expected for their age. A residual score of zero reflected that the individual was at the expected pubertal timing for their age.

Salivary cortisol. The seven passive drool samples collected at the sessions were placed in a freezer with a set temperature of -20 degrees Celsius (Davis et al., 2002). The samples were analyzed at the CORE Biomarker Lab at the The Pennsylvania State University with an expanded-range high-sensitivity enzyme immunosorbent assay kit (No. 1-3002/1-2012; Salimetrics, LLC, State College, PA). This kit detected cortisol levels that were within the range of 0.08-82.77 nmol/L in the saliva samples. These extractions were conducted twice.

Due to the different randomized coping conditions that participants completed following the administration of the TSST-C, cortisol values from saliva samples after the fourth sample were not included as part of the metric of stress reactivity (Figure 2). The fourth saliva sample that was collected after the coping condition was included because saliva cortisol levels are expected to reflect reactivity to situations 15-20 minutes prior to collection of the saliva sample. Thus, the fourth saliva sample is meant to measure the participant's response near the end of the TSST-C.

Internalizing problems. Parents reported on their children's internalizing problems on the Behavior Assessment System for Children, Second Edition (BASC-2; Reynolds & Kamphaus, 2004). The BASC-2 required parents to provide ratings on their child on 158 items. Parents identified whether each item was accurate in describing their child's behavior in the last several months using a Likert scale from 1 (never) to 4 (always). An internalizing problem subscale was derived from the behavior checklist, with α of 0.92 for the full sample and males only, and 0.91 for females only.

Potential covariates. Parent report of child's over-the-counter and prescription medication use, either regularly or taken the day of the session, was considered as a covariate. Each reported medication was scored on a Likert scale from 0 to 2 (0 = not plausible, 1 = possible, 2 = very plausible) on whether or not they could influence cortisol levels using the list compiled by Granger, Hibel, Fortunato, and Kapelewski (2009). The scores were summed to create a medication use variable. A two-sample t-test was conducted to examine whether

individuals' salivary cortisol reactivity significantly differed by medication use. Medication use did not influence cortisol reactivity, $t(138) = -0.60$, $p = .55$. Thus, medication use was not included in the final model.

Socioeconomic status (SES) was also considered as a covariate. Correlations were conducted to examine whether SES was significantly associated with any of the measures of interest. SES was not significantly correlated with interpersonal stress, internalizing problems, pubertal timing, or cortisol stress reactivity in the full sample ($ps > .05$) and was not ultimately included as a covariate in the final model.

Data analysis

Data reduction and preprocessing. The raw cortisol values for the four saliva samples that were used to assess physiological stress reactivity were winsorized by replacing values that were more than three standard deviations above or below the mean with the value of three standard deviations above or below the mean, respectively (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Wilcox, 1994). The raw cortisol values were then fourth-root transformed to address violations of assumptions of normality and homoscedasticity (Miller & Plessow, 2013). Cortisol stress reactivity was measured by the difference between the maximum and minimum transformed cortisol concentrations of the four selected samples (Miller et al., 2018). Recent work has shown that this method is more accurate in assessing cortisol stress reactivity than other traditional means (e.g., area under the curve, difference between last sample and baseline sample) (Miller et al., 2018).

Both the interpersonal stress and cortisol stress reactivity predictor variables were mean-centered for ease of analysis interpretation. The pubertal timing variable was already a residual score and did not require further mean-centering.

First-stage moderated mediation. Bootstrapped models reflecting the hypothesized mediation and moderation effects were conducted with the "processR" (Moon, 2019) and "lavaan" (Rosseel, 2017) packages in R. Each model consisted of regressions representing the hypothesized direct and indirect relationships between the variables as seen in Figure 1 (Washburn, n.d.). Models were fit using the SEM function in "lavaan" for the entire sample and separately for females and males. Indirect effects were assessed with bootstrapped bias-corrected confidence intervals (i.e., an indirect effect is significant if the corresponding confidence interval does not contain zero) (Hayes, Preacher, & Myers, 2011; Preacher & Hayes, 2008).

RESULTS

Descriptive statistics and correlations

Descriptive statistics of the sample are reported in Table 1. There were almost no significant differences by sex (*p*s > .05). Only reports of pubertal status on the PDS significantly differed between sexes, with females ($M = 1.96$) being reported as further along in pubertal development than males ($M = 1.44$), $t(124) = -6.12$, $p < .001$. Pubertal timing was not found to be significantly different between females ($M = 0.004$) and males ($M = 0.0005$), $t(124) = -0.04$, $p = .96$, further illustrating that both female and male participants on average are near the expected pubertal stage for their ages. Correlations of the variables of interest are presented in Table 2.

First-stage moderated mediation

Results are reported in the following section and presented in Figure 3.

Full sample. The overall model fit was mediocre, $\chi^2(4) = 22.50$, $p < .001$, CFI = 0.59, RMSEA = 0.19, 90% CI [0.12, 0.27], SRMR = 0.09. Interpersonal stressful events, $a_1 = -0.04$, SE = 0.03, $p = .28$, and salivary cortisol reactivity, $a_2 = -0.28$, SE = 0.48, $p = .56$, did not predict pubertal timing; however, there was a significant interaction of stressful events and cortisol reactivity when predicting pubertal timing, $a_3 = 1.17$, SE = 0.39, $p = .003$.

Hypothesis 1 (full sample): Though number of interpersonal stressful events did directly predict internalizing problems, such that more stressful events predicted increased reports of internalizing problems on the BASC, $c = 4.26$, SE = 0.80, $p < .001$, there was no sufficient evidence that pubertal timing predicted internalizing problems, $b = 1.37$, SE = 2.06, $p = .51$, indicating a lack of a mediation effect of pubertal timing.

Hypothesis 2 (full sample): Results demonstrated that there was no indirect effect of interpersonal stress on internalizing problems via pubertal timing that differed based on cortisol reactivity, $a_3b = -0.05$, SE = 0.12, $p = .65$. This was evident because a bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.47, 0.07]. Among participants who had lower salivary cortisol reactivity (1 SD below the mean cortisol reactivity value), there was no significant change in internalizing problems for every 1-unit decrease in the association between stressful events and pubertal timing, $a_1b + a_3b = -0.18$, SE = 0.29, $p = .53$. Similarly, there was no change in internalizing problems for every 1-unit decrease in the association between stressful events and pubertal timing among participants who had higher cortisol reactivity (1 SD above the mean cortisol reactivity value), $a_1b + a_3b = 0.07$, SE = 0.16, $p = .64$.

Females. The measurement model for females only also had mediocre fit, $\chi^2(4) = 11.05$, $p = .03$, CFI = 0.72, RMSEA = 0.17, 90% CI [0.05, 0.29], SRMR = 0.10. Total number of interpersonal stressful events did not predict earlier pubertal timing for females, $a_1 = -0.08$, SE = 0.05, $p = .12$, and salivary cortisol reactivity did not predict pubertal timing, $a_2 = -0.14$, SE = 0.95, $p = .88$. Similar to the full sample model, there was a significant interaction of stressful events and cortisol reactivity when predicting pubertal timing in females, $a_3 = 1.66$, SE = 0.64, $p = .009$.

Hypothesis 1 (females): Internalizing problems were directly and positively associated with interpersonal stressful events, $c = 4.67$, SE = 1.16, $p < .001$, but pubertal timing did not

predict internalizing problems in females, $b = 1.72$, SE = 2.34, $p = .46$. Pubertal timing did not mediate the relation between interpersonal stressful events and internalizing problems.

Hypothesis 2 (females): Among females, there was no evidence that an indirect effect of interpersonal stressful events on internalizing problems via pubertal timing differed based on salivary cortisol reactivity, $a_3b = -0.14$, SE = 0.23, $p = .56$. A bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.84, 0.18]. Among female participants who had lower salivary cortisol reactivity (1 SD below the mean cortisol reactivity value), there was no change in internalizing problems for every 1-unit increase in the association between stressful events and pubertal timing, $a_1b + a_3b = -0.35$, SE = 0.52, $p = .50$. Similarly, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing among female participants who had higher cortisol reactivity (1 SD above the mean cortisol reactivity value), $a_1b + a_3b = 0.08$, SE = 0.23, $p = .71$.

Males. The model fit for males only was poor, $\chi^2(4) = 12.62$, $p = .01$, CFI = 0.40, RMSEA = 0.19, 90% CI [0.08, 0.30], SRMR = 0.09. Unlike with the full sample and with females only, number of interpersonal stressful events, $a_1 = -0.003$, SE = 0.04, $p = .95$, salivary cortisol reactivity, $a_2 = -0.31$, SE = 0.53, $p = .57$, and the interaction between interpersonal stressful events and salivary cortisol reactivity, $a_3 = 0.56$, SE = 0.50, $p = .27$, did not predict pubertal timing.

Hypothesis 1 (males): Consistent with the previous models, internalizing problems were predicted by interpersonal stressful events, $c = 4.10$, SE = 0.99, $p < .001$, but were not predicted by pubertal timing, $b = 0.46$, SE = 5.13, $p = .93$, indicating that pubertal timing did not mediate the effect interpersonal stressful events had on internalizing problems.

Hypothesis 2 (males): There was no sufficient evidence that an indirect effect of interpersonal stressful events on internalizing problems via pubertal timing differed based on stress cortisol reactivity for males, $a_3b = -0.002$, SE = 0.19, $p > .99$. A bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.51, 0.34]. Among males who had lower salivary cortisol reactivity, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing, $a_1b + a_3b = -0.02$, SE = 0.35, $p = .95$. Similarly, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing among participants with higher salivary cortisol reactivity, $a_1b + a_3b = 0.02$, SE = 0.38, $p = .96$.

Hypothesis 3 (females vs. males): Because there was no moderated mediation effect on internalizing problems in females and males separately, the hypothesis that the model would be significant for females but not males was not supported.

Alternative models

Because the hypothesized statistical model yielded poor model fit across all individuals and within females and males, alternative models were explored.

Second-stage moderated mediation.

Perhaps there is a true moderated mediation model connecting childhood interpersonal stressful events, pubertal timing, cortisol stress reactivity, and internalizing problems; however,

the moderating effect of HPA stress reactivity may actually be on the relation between pubertal timing and internalizing problems (a second-stage moderation) rather than on the relation between childhood interpersonal stress events and pubertal timing (a first-stage moderation), as originally hypothesized. Thus, an additional moderated mediation model (Figure 4) was explored with the "processR" (Moon, 2019) and "lavaan" (Rosseel, 2017) R packages. Like the primary analyses, bootstrapped models were fit for the entire sample and then separately for females and males.

Full sample. The overall model fit for the second-stage moderated mediation was mediocre, $\chi^2(4) = 8.71$, $p = .07$, CFI = 0.81, RMSEA = 0.10, 90% CI [0.00, 0.19], SRMR = 0.06. Number of interpersonal stressful events did directly predict internalizing problems, such that more stressful events predicted increased reports of internalizing problems on the BASC, $c = 4.41$, SE = 0.81, $p < .001$. But there was no sufficient evidence that number of interpersonal stressful events predicted pubertal timing, $a = -0.03$, SE = 0.04, $p = .45$. Additionally, pubertal timing did not predict internalizing problems, $b_1 = 1.27$, SE = 2.17, $p = .56$. Salivary cortisol reactivity did not predict internalizing problems when adjusting for all other predictors, $b_2 = -7.70$, SE = 11.36, $p = .50$. There was also no significant interaction of pubertal timing and cortisol reactivity when predicting internalizing problems, $b_3 = -11.85$, SE = 35.89, $p = .74$.

Results demonstrated that there was no indirect effect of interpersonal stress on internalizing problems via pubertal timing that differed based on cortisol reactivity, $ab_3 = -0.04$, SE = 0.11, $p = .74$. This was evident because a bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.41, 0.09]. Among participants who had lower salivary cortisol reactivity (1 SD below the mean cortisol reactivity value), there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing, $ab_1 + ab_3 = -0.06$, SE = 0.18, $p = .73$. Similarly, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing among participants who had higher cortisol reactivity (1 SD above the mean cortisol reactivity value), $ab_1 + ab_3 = -0.01$, SE = 0.18, $p = .96$.

Females. The measurement model for females only had decent fit, $\chi^2(4) = 4.86$, $p = .30$, CFI = 0.93, RMSEA = 0.06, 90% CI [0.00, 0.21], SRMR = 0.06. Number of interpersonal stressful events directly predicted internalizing problems for females, such that more stressful events predicted increased reports of internalizing problems on the BASC, $c = 4.84$, SE = 1.23, $p < .001$. Total number of interpersonal stressful events did not predict pubertal timing for females, $a = -0.04$, SE = 0.06, $p = .49$, and pubertal timing did not in turn, predict internalizing problems, $b_1 = 1.53$, SE = 2.34, $p = .52$. Cortisol reactivity in response to the TSST did not independently predict internalizing problems, $b_2 = 0.49$, SE = 17.19, $p = .98$, and did not moderate the relationship between pubertal timing and internalizing problems, $b_3 = -15.85$, SE = 42.17, $p = .71$.

Among females, there was no evidence that an indirect effect of stressful events on internalizing problems via pubertal timing differed based on salivary cortisol reactivity, $ab_3 = -0.07$, SE = 0.19, $p = .73$. A bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.92, 0.11]. Among participants who had lower salivary cortisol reactivity (1 SD below the mean cortisol reactivity value), there was no change in internalizing problems for every 1-unit increase in the association between stressful events and pubertal timing, $ab_1 + ab_3 = -0.12$, SE = 0.32, $p = .71$. Similarly, there was no change in internalizing

problems for every 1-unit increase in the association between stressful events and pubertal timing among participants who had higher cortisol reactivity (1 SD above the mean cortisol reactivity value), $ab_1 + ab_3 = -0.01$, SE = 0.30, $p = .97$.

Males. The model fit for males only was overall mediocre, $\chi^2(4) = 5.87$, $p = .21$, CFI = 0.77, RMSEA = 0.09, 90% CI [0.00, 0.22], SRMR = 0.07. Like with the full sample and with females only, number of interpersonal stressful events directly predicted higher levels of internalizing problems, $c = 4.27$, SE = 1.02, $p < .001$. When the model was run for just males, there was no indication that interpersonal stressful events significantly predicted pubertal timing, $a = -0.007$, SE = 0.03, $p = .82$. Additionally, pubertal timing did not predict internalizing problems, $b_1 = -0.24$, SE = 5.25, $p = .96$. Salivary cortisol reactivity did not predict internalizing problems after adjusting for all other predictors, $b_2 = -16.15$, SE = 18.90, $p = .39$. Pubertal timing and cortisol stress reactivity also did not significantly interact in predicting internalizing problems, $b_3 = -36.05$, SE = 85.17, $p = .67$.

There was no sufficient evidence that an indirect effect of interpersonal stressful events on internalizing problems via pubertal timing differed based on stress cortisol reactivity for males, $ab_3 = 0.001$, SE = 0.17, $p > .99$. A bias-corrected bootstrapped confidence interval for indirect effect included zero, 95% CI [-0.38, 0.39]. Among males who had lower salivary cortisol reactivity, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing, $ab_1 + ab_3 = -0.02$, SE = 0.28, $p = .94$. Similarly, there was no change in internalizing problems for every 1-unit increase in the association between interpersonal stressful events and pubertal timing among participants with higher salivary cortisol reactivity, $ab_1 + ab_3 = 0.02$, SE = 0.32, $p = .94$.

Multiple mediation

Another alternative approach to better understanding what explains the association between stress and internalizing psychopathology is to conceptualize both pubertal timing and HPA stress reactivity as mediators. Trickett and Putnam's (1993) theory regarding sexual abuse predicting mental health outcomes implicates both the hypothalamic-pituitary-gonadal (HPG) and HPA axes as physiological systems that can be impacted by stress exposure and subsequently impact outcomes. It is possible that the two systems, measured by pubertal timing and cortisol stress reactivity respectively, have separate effects on the relation between childhood interpersonal stress and internalizing problems. A bootstrapped multiple mediation model was tested with the R "lavaan" package (Rosseel, 2017). Figure 5 depicts the modeled separated mediation effects of pubertal timing and cortisol stress reactivity in the full sample, females only, and males only.

Full sample. The overall model fit for the multiple mediation in the full sample was good, $\chi^2(6) = 26.42$, $p < .001$, CFI = 1.00, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR = 0.00. Number of interpersonal stressful events did directly predict internalizing problems, such that more stressful events predicted increased reports of internalizing problems on the BASC, $c = 4.32$, SE = 0.79, $p < .001$; however, there was no sufficient evidence that number of stressful events predicted pubertal timing, $a_1 = -0.03$, SE = 0.04, $p = .44$, or cortisol stress reactivity, $a_2 = 0.008$, SE = 0.007, $p = .29$. Pubertal timing, $b_1 = 1.43$, SE = 2.20, $p = .52$, and cortisol stress reactivity, $b_2 = -8.07$, SE = 11.20, $p = .47$, also did not subsequently predict internalizing problems.

There was no indirect effect of interpersonal stress on internalizing problems via pubertal timing, a_1b_1 = -0.04, SE = 0.11, p = .73. Additionally, there was no indirect effect of interpersonal stress on internalizing problems via cortisol stress reactivity, a_2b_2 = -0.06, SE = 0.13, p = .62. Bias-corrected bootstrapped confidence intervals for indirect effects of pubertal timing, 95% CI [-0.46, 0.07], and cortisol stress reactivity, 95% CI [-0.47, 0.07], included zero.

Females. The overall model fit for the multiple mediation for females only was also good, $\chi^2(6) = 19.32$, p = .004, CFI = 1.00, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR < 0.001. Number of interpersonal stressful events directly predicted internalizing problems, such that more stressful events predicted increased reports of internalizing problems on the BASC, c = 4.66, SE = 1.12, p < .001. Number of interpersonal stressful events did not predict either pubertal timing, a_1 = -0.04, SE = 0.06, p = .45, or cortisol stress reactivity, a_2 = 0.01, SE = 0.01, p = .28. Pubertal timing, b_1 = 1.72, SE = 2.40, p = .47, and cortisol stress reactivity, b_2 = 0.24, SE = 16.54, p = .99, also did not subsequently predict internalizing problems.

Similar to findings for the full sample, there was no indirect effect of interpersonal stress on internalizing problems via pubertal timing, a_1b_1 = -0.08, SE = 0.19, p = .70, in females only. Additionally, there was no indirect effect of interpersonal stress on internalizing problems via cortisol stress reactivity, a_2b_2 = 0.003, SE = 0.23, p = .99. Bias-corrected bootstrapped confidence intervals for indirect effects of pubertal timing, 95% CI [-0.86, 0.10], and cortisol stress reactivity, 95% CI [-0.43, 0.58], included zero.

Males. The overall model fit for the multiple mediation for males only was good, $\chi^2(6) = 11.17$, p = .08, CFI = 1.00, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR < 0.001. Like with the full sample and females only, number of interpersonal stressful events directly predicted internalizing problems, c = 4.17, SE = 0.95, p < .001, but not pubertal timing, a_1 = -0.007, SE = 0.04, p = .83, or cortisol stress reactivity, a_2 = 0.004, SE = 0.01, p = .67. Internalizing problems was also not predicted by pubertal timing, b_1 = 0.35, SE = 5.10, p = .95, or cortisol stress reactivity, b_2 = -17.43, SE = 17.97, p > .33.

Pubertal timing, a_1b_1 = -0.003, SE = 0.19, p = .99, and cortisol stress reactivity, a_2b_2 = -0.08, SE = 0.25, p = .76, did not have indirect effects on the relation between interpersonal stress and internalizing problems. This was confirmed by bias-corrected bootstrapped confidence intervals for indirect effects of pubertal timing, 95% CI [-0.43, 0.42], and cortisol stress reactivity, 95% CI [-0.93, 0.22], that included zero.

Multiple-step, multiple mediation

Instead of pubertal timing and cortisol stress reactivity representing separate constructs of pubertal development and HPA functioning, they might actually be part of a chain effect in which pubertal development causes changes in the HPA axis. Gunnar and colleagues (2009; 2019) have found that cortisol stress reactivity naturally changes over time, specifically during adolescence when pubertal development also occurs. There may be a sequential effect of stressful life events on internalizing problems through a causal relationship between pubertal timing and cortisol stress reactivity. Figure 6 depicts a multiple-step, multiple mediator model that was tested with the "lavaan" package in R in order to examine a potential chain effect from interpersonal stress exposure to pubertal development to physiological stress reactivity and ultimately to internalizing problems.

Full Sample. The overall model fit for the multiple-step, multiple mediation in the full sample was good, $\chi^2(6) = 26.42$, $p < .001$, CFI = 0.98, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR = 0.02. As with all previously run models, interpersonal stressful life events positively predicted internalizing problems, $c = 4.28$, SE = 0.78, $p < .001$. Cortisol stress reactivity did not significantly predict internalizing problems, $b = -7.72$, SE = 10.71, $p = .47$. Additionally, neither interpersonal stressful events, $a_2 = 0.008$, SE = 0.007, $p = .25$, nor pubertal timing, $d = 0.02$, SE = 0.02, $p = .47$, predict cortisol stress reactivity. Pubertal timing was not predicted by interpersonal stressful events, $a_1 = -0.03$, SE = 0.04, $p = .44$.

There were no indirect effects of a chain reaction through pubertal timing and cortisol stress reactivity, $a_1db = 0.003$, SE = 0.02, $p = .86$, bias-corrected 95% CI [-0.004, 0.12], or through cortisol stress reactivity by itself, $a_2b = -0.06$, SE = 0.12, $p = .60$, bias-corrected 95% CI [-0.53, 0.08], on internalizing problems in the full sample.

Females. Model fit for the model was good for females only, $\chi^2(6) = 19.32$, $p = .004$, CFI = 0.96, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR = 0.03. Interpersonal stressful life events positively predicted internalizing problems, $c = 4.58$, SE = 1.16, $p < .001$. Cortisol stress reactivity did not significantly predict internalizing problems, $b = 1.39$, SE = 16.66, $p = .93$. Additionally, neither interpersonal stressful events, $a_2 = 0.01$, SE = 0.01, $p = .33$, nor pubertal timing, $d = -0.01$, SE = 0.05, $p = .82$, predict cortisol stress reactivity. Pubertal timing was not predicted by interpersonal stressful events, $a_1 = -0.04$, SE = 0.06, $p = .45$.

There were no indirect effects of a chain reaction through pubertal timing and cortisol stress reactivity, $a_1db = 0.001$, SE = 0.10, $p > .99$, bias-corrected 95% CI [-0.08, 0.25], or through cortisol stress reactivity by itself, $a_2b = 0.01$, SE = 0.26, $p = .96$, bias-corrected 95% CI [-0.47, 0.65], on internalizing problems in the full sample.

Males. Model fit for the model was also good when testing with just males, $\chi^2(6) = 11.17$, $p = .08$, CFI = 1.00, RMSEA < 0.001, 90% CI [0.00, 0.00], SRMR = 0.003. Interpersonal stressful life events positively predicted internalizing problems, $c = 4.17$, SE = 0.92, $p < .001$. Cortisol stress reactivity did not significantly predict internalizing problems, $b = -17.47$, SE = 17.67, $p = .32$. Additionally, interpersonal stressful events, $a_2 = 0.003$, SE = 0.01, $p = .83$, predicted cortisol stress reactivity. But pubertal timing did predict cortisol stress reactivity such that earlier pubertal timing was associated with less physiological reactivity, $d = -0.22$, SE = 0.03, $p < .001$. Pubertal timing was not predicted by stressful events, $a_1 = -0.007$, SE = 0.03, $p = .82$.

There were no indirect effects of a chain reaction through pubertal timing and cortisol stress reactivity, $a_1db = -0.03$, SE = 0.20, $p = .89$, bias-corrected 95% CI [-0.69, 0.25], or through cortisol stress reactivity by itself, $a_2b = -0.05$, SE = 0.34, $p = .89$, bias-corrected 95% CI [-1.21, 0.39], on internalizing problems in the full sample.

Hierarchical multiple regression

Finally, hierarchical multiple regression analysis was used to assess individual contributions of interpersonal stressful events, pubertal timing, and cortisol stress reactivity on internalizing problems while accounting for effects of the other variables along with age as a covariate. For the full sample, individual interactions with sex for each of the variables of interest were examined as well. Age was centered based on the mean of the sample in question for each set of analyses. Sex (0 = males; 1 = females) was dummy coded. Models were tested

with the "stats" package in R. Table 3 presents the results for the series of analyses conducted in the full sample. Tables 4 and 5 display the results for sex-specific hierarchical multiple regression analyses.

Model 1. Within the full sample, the first model examined whether age and sex predicted internalizing problems. Neither age nor sex were predictive of internalizing problems, *p*s > .05. Within females only and males only, age as a covariate also did not significantly account for internalizing problems, *p*s > .05.

Model 2. The addition of stressful life events in Model 2 significantly increased R^2 in all three models, *p*s < .01. Interpersonal stress positively predicted internalizing problems in the full sample, $b = 4.16$, SE = 1.28, $p = .001$, in females only, $b = 4.63$, SE = 1.11, $p < .001$, and in males only, $b = 4.18$, SE = 1.30, $p = .002$. In the full sample, sex did not interact with stressful life events to predict internalizing problems, $b = 0.50$, SE = 1.69, $p = .77$.

Model 3. The addition of pubertal timing as a predictor of internalizing problems in Model 3 did not significantly increase R^2 in all three models, *p*s > .05. Pubertal timing did not predict internalizing problems after accounting for effects of covariates and interpersonal stressful life events, *p*s > .05. This was the case for the full sample, $b = 0.59$, SE = 4.18, $p = .89$, females, $b = 2.02$, SE = 2.46, $p = .42$, and males, $b = 0.60$, SE = 4.24, $p = .89$. Sex also did not interact with pubertal timing to predict internalizing problems within the full sample, $b = 1.50$, SE = 4.83, $p = .76$; however, there was a main effect of sex on internalizing problems with the addition of pubertal timing as a predictor, such that females had more internalizing problems than males, $b = 4.00$, SE = 1.85, $p = .03$.

Model 4. Similarly, the addition of cortisol stress reactivity to the TSST as another predictor of internalizing problems, did not greatly increase R^2 from Model 3 to Model 4, *p*s > .05. Cortisol stress reactivity did not significantly predict internalizing problems in the full sample, $b = -17.33$, SE = 15.80, $p = .28$, in females separately, $b = 2.16$, SE = 17.70, $p = .90$, and in males separately, $b = -17.32$, SE = 15.96, $p = .28$. There was no interaction between cortisol stress reactivity and sex in the full sample, $b = 19.84$, SE = 23.57, $p = .40$. Like with the previous model, there was a main effect of sex on internalizing problems, such that females had more internalizing problems than males, $b = 4.08$, SE = 1.86, $p = .03$.

DISCUSSION

The aim of the current study was to examine a model of the relationship between interpersonal stress and mental health problems in the context of pubertal development and HPA stress reactivity during early adolescence. This was accomplished with a first-stage moderated mediation model with three accompanying hypotheses. Hypothesis 1 was that interpersonal stress's positive effect on internalizing problems would be mediated by earlier pubertal timing. Results suggest that interpersonal stressful events are directly and positively associated with internalizing problems but that the mechanism through which this occurs may not be via pubertal timing; these results do not support Hypothesis 1. Hypothesis 2 predicted that HPA stress hyperreactivity via salivary cortisol levels moderated the mediation effect of earlier pubertal timing. Because there was no mediation via pubertal timing to be moderated by cortisol stress reactivity, Hypothesis 2 was not supported. Hypothesis 3 predicted that the overall first-stage moderated mediation model would only be significant for females and not males. Hypothesis 3 was not supported, as the moderated mediation model was not a good fit for either sex. The only significant sex difference in the tested relationships included in the model was in how the interaction between interpersonal stressful events and cortisol stress reactivity predicted pubertal timing in the full sample and in females but not in males. That there was no significant sex difference in internalizing problems may explain why the rest of the first-stage moderated mediation model was not different between females and males.

Because the sample was comprised of early adolescents, it is possible that the emergence of sex-differentiated risk for internalizing psychopathology had not occurred yet. Though there was a sex difference in pubertal status (females were reported to be more advanced in pubertal development in males), this was expected because participants were recruited by age and not pubertal status. Females on average have been shown undergo puberty earlier than males, so females in this sample are expected to be further along in puberty than their same-aged male peers (Marceau, Ram, Houts, Grimm, & Susman, 2011; Susman et al., 2010). Additionally, females and males in this sample did not differ in pubertal timing, which was hypothesized to contribute to internalizing problems. Thus, the sex difference in pubertal status as measured by the PDS was, as expected, not associated with internalizing problems.

Stress: the origin of negative outcomes

The present study found that there was a direct association of interpersonal stress on internalizing problems such that the more stressful events reported, the more internalizing problems are reported, regardless of sex. All alternative models examined also supported this. This finding supports previous literature that links childhood stress to psychopathology (e.g., McLaughlin et al., 2010) as well as theory regarding specifically interpersonal stress as contributing to internalizing psychopathology (Cicchetti & Toth, 1998; Hammen, 1992).

This may be explained by alterations to biological systems such as the neuroendocrine system (Penza, Heim, & Nemeroff, 2003) through a calibration effect that adjusts the physiological stress response during childhood and primes it to activate in response to stressful experiences during adulthood (ACM; Del Giudice et al., 2011). Other theories regarding stress effects on outcomes also assume that there is some biological mechanism in play, potentially via pubertal processes (Belsky et al., 1991) or physiological stress reactivity processes (Trickett & Putnam, 1993). Results from this study found some evidence for these theories; interpersonal stress exposure and stress reactivity interacted to predict differences pubertal timing in females.

For females who were exposed to fewer interpersonal stressful events, cortisol hyporeactivity predicted earlier pubertal timing. For females who were exposed to more interpersonal stressful events, cortisol hyperreactivity predicted earlier pubertal timing. This, in light of prior evidence that exposure to stress is related to cortisol hyperreactivity in females (Bosch et al., 2012), that cortisol hyperreactivity is associated with internalizing problems when females are more advanced in pubertal development (Colich et al., 2015), and that HPA stress reactivity as moderator on association between stress and behavioral outcomes (Hagan et al., 2014; Steeger et al., 2017), suggests that interpersonal stress and HPA hyperreactivity both influence pubertal timing and potentially indirectly go on to influence the presence of internalizing problems via earlier pubertal timing.

The overall connection of stress to negative outcomes specifically via these physiological processes was not supported by our findings. The positive association between interpersonal stress and internalizing problems was not mediated by earlier pubertal timing. Therefore, a mediation effect of pubertal timing was not moderated by cortisol stress reactivity so that the mediation was stronger for individuals with cortisol hyperreactivity. When referring back to theories driving this study, it is important to remember that many of the proposed models of biological mechanisms underlying the association between stress and outcomes is in the context of specific and/or high-risk environments. Belsky et al.'s (1991) theory focuses on a normative range of stressors within the family and home context. The interpersonal items of the stress measure used in this study (CASE-P) also might also count stressors occurring in other domains, such as peer and school. Trickett and Putnam's (1993) theory was specifically about experiences of childhood sexual abuse, something that was not measured in our sample. Thus, the nature of the interpersonal stressors assessed in the current study did not match the nature of the stressors implicated by existing theory and potentially contributed to overall null findings.

Another possible mismatch between the risk level of our sample and that assumed by existing theory could also explain why the entire first-stage moderated mediation model was not different by sex. Though separate interpersonal stress effects on pubertal timing and internalizing problems were found in females, there was no evidence to suggest that pubertal timing, as reported by parents, directly affected internalizing problems. As suggested by the ACM, females may be more physiologically responsive to stress, and thus, physiological processes like increased HPA stress reactivity and earlier pubertal maturation may help explain the female-driven positive association between interpersonal stressful life events and internalizing problems. Though the ACM suggests that sex differences in stress response (which could go on to contribute to development of psychopathology) occur due to differences in the nature of the biological calibration (Del Giudice et al., 2011), the present study's results do not support this notion. Additionally, sex differences in which females are predicted to be withdrawn while males are expected to be agonistic (Del Giudice et al., 2011) in the face of a dangerous and/or unpredictable environment may not be seen in a community sample of children who may not have all been exposed to such an environment or to stressors of high severity. This may also explain why the hypothesized model was not a good fit with the data.

But that there was a sex difference at all in how interpersonal stress predicted pubertal timing via an interaction with cortisol stress reactivity supports previous literature and theory that exposure to stress, at least stress of an interpersonal nature, has more of an influence on pubertal development in females. Additionally, the lack of a significant interaction of interpersonal stressful life events and cortisol stress reactivity in predicting pubertal timing in males is further evidence that pubertal development in males may not necessarily be influenced

by constructs like interpersonal stress. Generally, findings regarding the relation between stress and pubertal timing suggest that the association is significant in females and not in males (Belsky et al., 2010). This may be driven by the relative ease of measuring pubertal timing via age of menarche in females versus a lack of a clear marker of pubertal development in males. Results from the current study serve to support the notion that puberty's role in influencing the relation between interpersonal stress and outcomes may truly be more relevant for females than males.

Pubertal timing and HPA stress reactivity—Two sides of the same coin?

The present study attempted to explore potential biological mechanisms through which internalizing problems develop from exposure to interpersonal stress during childhood. Pubertal timing and HPA stress reactivity were considered as plausible candidates due to their respective associations with stress and internalizing psychopathology in the literature. The overall first-stage moderated mediation model was based on the psychosocial acceleration theory that posits that pubertal timing is affected by childhood stress and is actually the mechanism through which maladaptive behaviors and psychopathology develop (Belsky et al., 1991). The tested model added salivary cortisol reactivity to stress as a key physiological influence on this mechanism. This was based on previous work finding that HPA stress reactivity can moderate the relation between stress and psychopathology (Hagan et al., 2014; Steeger et al., 2017). Cortisol stress reactivity did moderate the relation between interpersonal stress and pubertal timing in females, specifically; however, model fit was generally mediocre, and null results were found for a mediating effect of pubertal timing on the association between stress and internalizing problems in the full sample as well as separately in females and males.

Thus, a first alternative model was tested in which a second-stage moderated mediation was hypothesized to better fit the data. According to this alternative model, cortisol stress reactivity may actually play a role in the process by moderating pubertal timing effects on internalizing problems. Like the originally hypothesized first-stage moderated mediation, model fit was generally mediocre, save for females only. That this model had decent fit for female only data suggests that a second-stage model may be a better fit in explaining how interpersonal stress, internalizing problems, pubertal timing, and HPA stress reactivity are related in female early adolescents. Overall, this first alternative model was consistent with the original first-stage model in showing that interpersonal stress and internalizing problems are associated, but it did not provide support for influences of HPA functioning or pubertal development on this relation.

The first- and second-stage moderated mediation models tested assumed that pubertal timing plays a pivotal role in generating the link between interpersonal stress and internalizing problems while HPA axis functioning does not have an influence; however, as suggested by Trickett and Putnam's (1993) sexual abuse model, stress exposure may separately affect pubertal and HPA development, which each go on to affect internalizing problems. Thus, a second alternative model was tested via multiple mediation of both pubertal timing and cortisol stress reactivity. This multiple mediation model fit the data better than the first- and second-stage moderated mediation models, suggesting that cortisol stress reactivity actually does play a mediating rather than moderating role in the emergence of internalizing psychopathology from stress exposure; however, like the second-stage moderated mediation model, this model only found that interpersonal stressful life events predicted internalizing problems for both sexes.

Pubertal timing and HPA stress reactivity were treated as separate entities in the originally hypothesized model, the second-stage alternative, and the multiple mediation alternative; however, there is existing evidence that suggests that the two constructs are

intrinsically linked and that the development of HPA regulation in response to stress may actually be dependent on pubertal timing or vice versa. Physiological stress reactivity as measured by salivary cortisol seems to change across pubertal development without any intervention (Gunnar et al., 2009; Gunnar et al., 2019). There is a possibility that the fact that cortisol stress reactivity naturally changes as puberty progresses is not a coincidence and that one may cause the other through a chain effect. A chain effect of advanced pubertal status directly causing cortisol hyperreactivity may explain the original model's null findings, because the tested model did not consider a causal link between pubertal timing and HPA axis functioning but rather assumed that the latter only influenced the former's effects on internalizing problems. By taking into consideration the possibility that HPA axis functioning is dependent on pubertal development based on previous findings that HPA functioning naturally changes across time (Gunnar et al., 2009; Gunnar et al., 2019), perhaps the path from interpersonal stress to internalizing symptoms is indeed through early pubertal timing, as hypothesized. But rather than early pubertal timing directly affecting psychopathology, early timing actually leads to increased HPA stress reactivity, which then serves to directly trigger the development of internalizing symptoms. This was assessed cross-sectionally with the fourth alternative model in the form of a multiple-step, multiple mediation, with a chain effect going through interpersonal stress, pubertal timing, cortisol stress reactivity, and internalizing problems in order. Results from the testing of this model were similar to that of testing of the other models in that only the direct effect of interpersonal stress on internalizing problems was consistently significant. Additionally, like with most of the other models tested, there were no direct or indirect effects of pubertal timing and cortisol stress reactivity on the mental health outcome. This may be due to testing the multiple-step, multiple mediation in cross-sectional data. Interestingly, pubertal timing was significantly associated with cortisol stress reactivity for males but not for females in this model. Though this is contrary to expectations that pubertal timing has more influence on outcomes in females than in males (e.g., Belsky et al., 1991), it is important to note that interpretation of effects in this model is difficult due to its cross-sectional nature. Testing this model longitudinally with pubertal timing during early adolescence and cortisol stress reactivity during mid-adolescence may better reflect the potential causal relationship of puberty on HPA axis functioning.

Given that the main model and aforementioned alternative models were all structural equation models (SEMs) and interpretation of their results is limited due to the study's relatively small sample size, a hierarchical multiple regression model was tested to better understand whether interpersonal stress, pubertal timing, and cortisol stress reactivity individually contributed to internalizing problems. Consistent with the structural equation models, interpersonal stress was consistently significantly associated with internalizing problems in the full sample as well as separately in females and males while pubertal timing and cortisol stress reactivity each had minimal effect; however, females were shown to have more internalizing problems than males in the regression models that included pubertal timing and cortisol reactivity. This could be evidence that there is indeed a sex difference in internalizing problems, but it is caused by a construct that is not explained by pubertal timing or HPA stress reactivity.

Limitations

There are several limitations to the current study that might have contributed to the findings. First, the cross-sectional nature of the study may not necessarily be the best design to test the hypothesized relationships between interpersonal stressful events, pubertal timing,

salivary cortisol reactivity, and internalizing problems. Concurrently measuring internalizing problems with childhood stressful events does not allow for investigation of mechanisms that may take time to develop the relations between stress and behavioral outcomes, especially if they are influenced by biological processes that take considerable amounts of time, such as puberty (Susman, Marceau, Dockray, & Ram, 2019) and the development of the HPA axis throughout adolescence (Gunnar et al., 2009).

Additionally, the use of the CASE-P to capture number of interpersonal stressful events may not be the most accurate method to measure interpersonal stress. The interpersonal stress score was determined by the cumulative number of interpersonal events endorsed on the CASE-P. This method did not consider the individual severity and subjective emotional valence of each interpersonal life event the child had experienced. For example, one participant could have experienced one subjectively negative event that had an impact for a long period of time (e.g., months of intense physical and emotional bullying by an older child) while another participant could have experienced several events that were individually not as severe and had shorter term effects (e.g., three instances of light teasing by three different friends). The second participant would have had a higher stress score than the first participant, even though their subjective experiences would suggest the opposite. The CASE-P also did not have a very wide range of possible interpersonal events for participants to endorse, leaving the possibility that there were many interpersonal life events that were not accounted for in the sample.

It is also imperative to note that the measure of stressful events in the present study is a measure of general interpersonal stress rather than interpersonal stress in specific domains. Pubertal timing has most commonly been associated with stressors that are either specifically tied to the family context (i.e., father absence, harsh parenting) or to more traumatic stressors (i.e., sexual abuse). These findings support theory that the stressors that are most salient in predicting one's environment and subsequent reproductive success are the ones that go on to influence pubertal timing. Though some interpersonal stressors (e.g., having an argument with a family member) may fall into this category, many others may not (e.g., breaking up with a romantic partner). The tested first-stage moderated mediation model may have yielded more significant results if the investigation of stress was more specific to types of interpersonal stressors that are linked to pubertal timing.

Pubertal timing was determined via parent-report of their children's current pubertal development on the PDS, which could be a limitation to the present study's goal of examining whether pubertal timing mediated stress effects on internalizing problems. Parent-report on the PDS has relatively low absolute agreement with other forms of measuring puberty (e.g., $\kappa = .28$ when comparing to clinician-rated Tanner staging (Koopman-Verhoeff, Gredvig-Ardito, Barker, Saletin, & Carskadon, 2020). Additionally, parents' reports of their children's pubertal status generally range in low to moderate agreement with examiner ratings (i.e., κs = .13-.55; Dorn et al., 1990). Dorn and Biro (2011) point out that physical examination conducted by a trained clinician is the current best way to determine pubertal status. Another method many other studies have used to measure pubertal status is to obtain self-report with ratings informed by pictures such as those depicted by the Tanner Staging questionnaire (LeMoult et al., 2019). In the case of the present study, having a parent rate their child's pubertal staging during this early adolescent period limits the assessment of pubertal development to the parent's observations of their child and what they might hear from their child's own report. Additionally, that the sample consisted of mostly mothers reporting on their children's pubertal status may have contributed to low internal consistency ($\alpha = 0.28$) in the pubertal status score for males. This is supported by

literature that finds that mothers are more accurate in reporting their daughters' development than in reporting their sons' development (Brooks-Gunn et al., 1987; Dorn et al., 1990). Thus, the derived pubertal timing measure that was ultimately used in the model may not have been an accurate assessment of males' pubertal timing.

Though parent-report on the PDS may not be the most accurate assessment of children's true pubertal status, the most proper measure to use ultimately depends on the research question. The current study is concerned with pubertal timing in the context of exposure to interpersonal stressors that can lead to behavior problems. Earlier pubertal timing in females is thought to place them at higher risk for psychological problems than their peers who are on-time or are late maturers, potentially through perceived differences between them and their peers and related social changes that they are not cognitively ready to confront (Ge, Conger, & Elder, 1996; Mendle et al., 2007). Thus, a female's perceived earlier pubertal timing can put them at higher risk for exposure to interpersonal stress, making them more vulnerable to developing internalizing problems. Therefore, in the case of the current study, others' (i.e., parents') perceptions of an individual's earlier pubertal timing may actually be more relevant for the link between interpersonal stress and internalizing problems than the individual's true pubertal status.

Regardless of whether parent-report was more appropriate to test the current study's hypothesized model, another limitation of the current study was that pubertal timing was derived through a single measure of pubertal status at one timepoint. Utilization of longitudinal methods to measure pubertal timing, such as linear models (Mendle, Harden, Brooks-Gunn, & Graber, 2010), latent transition analyses (Belsky et al., 2007), and growth curve models (Beltz, Corley, Bricker, Wadsworth, & Berenbaum, 2014), would offer a more detailed view into the timing of onset of puberty in instead of limiting the measure of pubertal timing to a snapshot of a singular point during development. The timing of puberty in particular could either play a role in the development of an individual's physiological reactivity to stress or function as an indicator for variations from typical HPA responsivity (Joos et al., 2018; Smith & Powers, 2009).

Finally, the current study's sample size (63 males and 63 females) used for a model with four variables limited statistical power. Rules of thumb for SEMs like moderated mediations vary greatly and range from a suggested 10 participants for each variable (Schreiber, Nora, Stage, Barlow, & King, 2006) to a sample size of at least 100-200 (Boomsma, 1982). Though the current study's sample and hypothesized moderated mediation model falls within this range of suggestions, effect sizes of the different inter-relations in the model were mostly small for both females and males (ds = .01-.55). Small effects like that of the current study require larger sample sizes in order to be detected as significant. The Monte Carlo simulation method (Muthén & Muthén, 2002) is the recommended way to determine a sample size that will yield adequate power for an SEM while accounting for both direct and indirect effects as well as other important model parameters (Wolf, Harrington, Clark, & Miller, 2013). A future study investigating inter-relations between interpersonal stress, internalizing problems, pubertal timing, and cortisol stress reactivity should use this method, which allows for testing of the hypothesized model in a wide range of sample sizes, when deciding on an appropriate sample size.

Future directions

It is imperative to continue exploring potential effects of childhood stress on mental health outcomes. In order to do so, future investigations on the potential relationships between stress and psychological problems should take steps to ensure that measurement of stress is comprehensive. Measures of childhood stress that would be potentially related to pubertal timing

should aim to take a more nuanced approach to operationalizing stress. As previously mentioned, a sum of reported stress events may not adequately capture the severity of stress in the last year. Using weighted measurements of endorsements of stress based on severity would be a logical next step in order to better understand stress effects on outcomes. Furthermore, special attention should be placed on interpersonal stress that occurs in the family context as well as more severe trauma.

In addition, pubertal timing should continue to be considered in investigations on stress effects on outcomes in late adolescence and adulthood. It is clear that puberty plays a large role in physical and cognitive development throughout adolescence. The question remains whether pubertal timing is the mechanism through which stress is linked to outcomes as well. Moreover, focus should be placed on why pubertal timing in many studies in the literature has been found to be influential on behavioral and mental health outcomes for females but not males. Examining the specific mechanisms through which pubertal timing mediates the relationship between stress and behavioral outcomes is a potential way to shed light on why increased exposure to stress and early pubertal timing are individually and consistently linked to negative outcomes.

The hypothesized processes of how internalizing problems emerge via pubertal and interpersonal stress mechanisms likely take time and occur across development, making it difficult to fully capture with cross-sectional methods. Thus, repeated measures and longitudinal testing of this present study's proposed model and alternatives is imperative to provide a more comprehensive perspective of what mechanisms enable the development of internalizing psychopathology. This is especially relevant given that early life and childhood stress is thought to set the stage for negative outcomes that become noticeably different by sex during adolescence and persist across the lifecourse.

Conclusion

Though the present study had limitations that prevented further interpretation of the hypothesized first-stage moderated mediation effect of pubertal timing and stress cortisol reactivity on internalizing problems, the resulting findings contribute to literature that emphasizes the important role childhood stress has on outcomes. It remains to be seen if this relationship is potentially sex-differentiated through pubertal development and stress biology, especially when it comes to internalizing psychopathology such as depression and anxiety. Currently, existing evidence suggests that this is the case, as internalizing disorders are strongly linked to interpersonal stress exposure in females more than in males. Continued consideration of potential biological mechanisms that contribute to the divergence of prevalence of related symptoms in females versus males is necessary. Investigations of pubertal development and HPA axis functioning starting in adolescence will aid in the determination of the most appropriate prevention and treatment modalities for adolescent-onset internalizing psychopathology.

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APPENDIX A

Figure 1

Conceptual First-Stage Moderated Mediation Model of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems

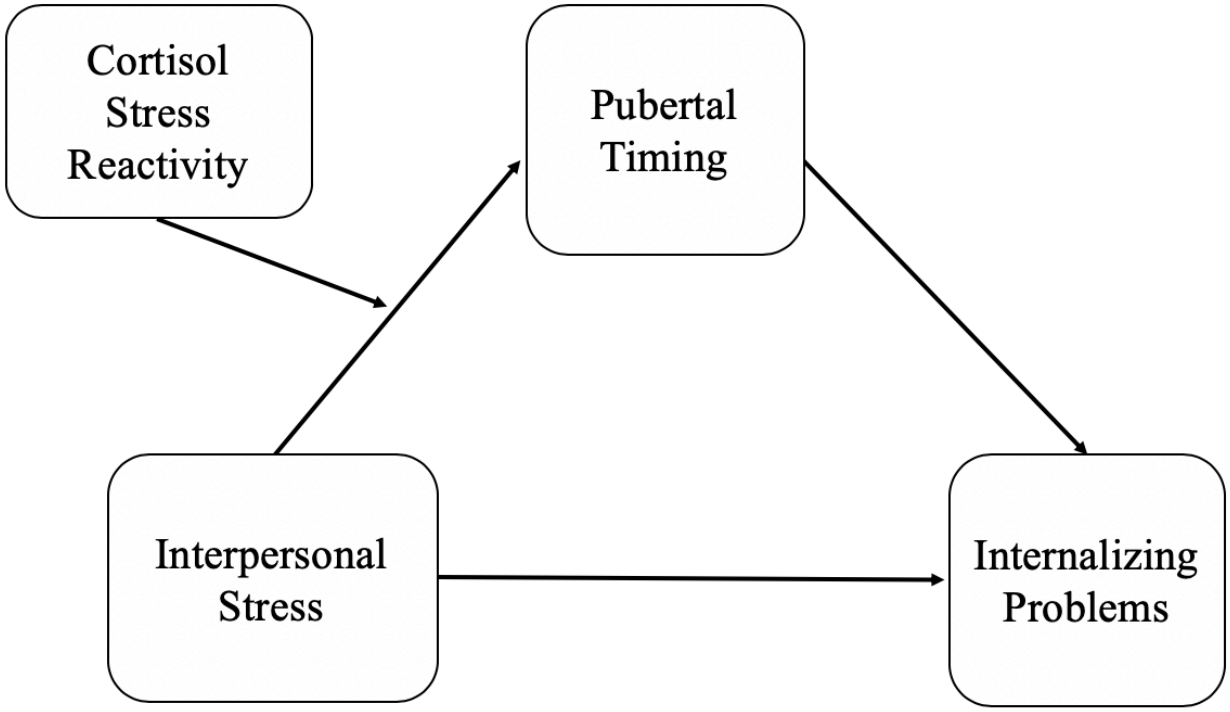
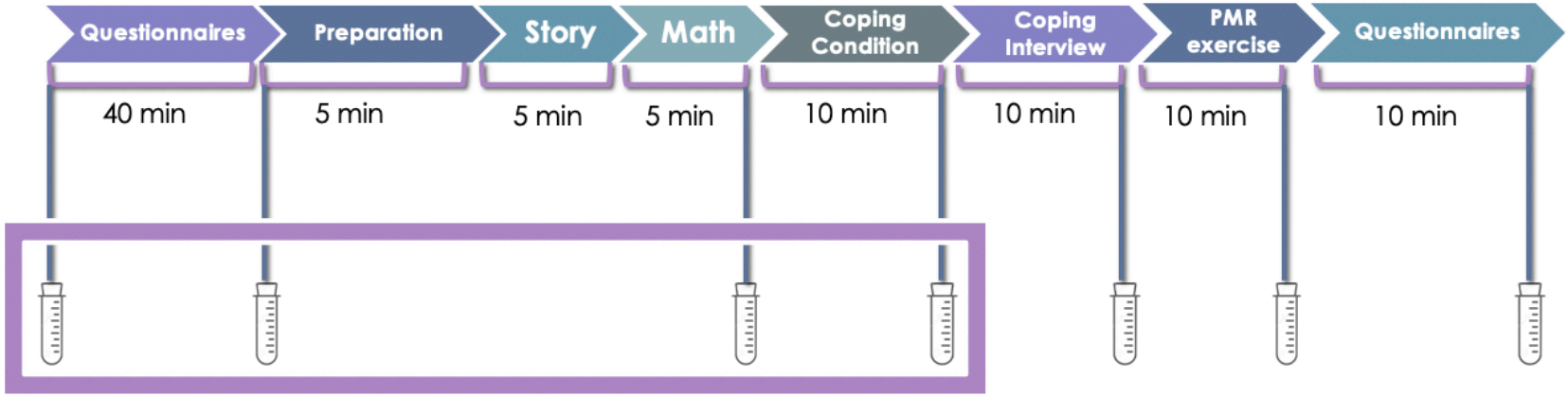


Figure 2

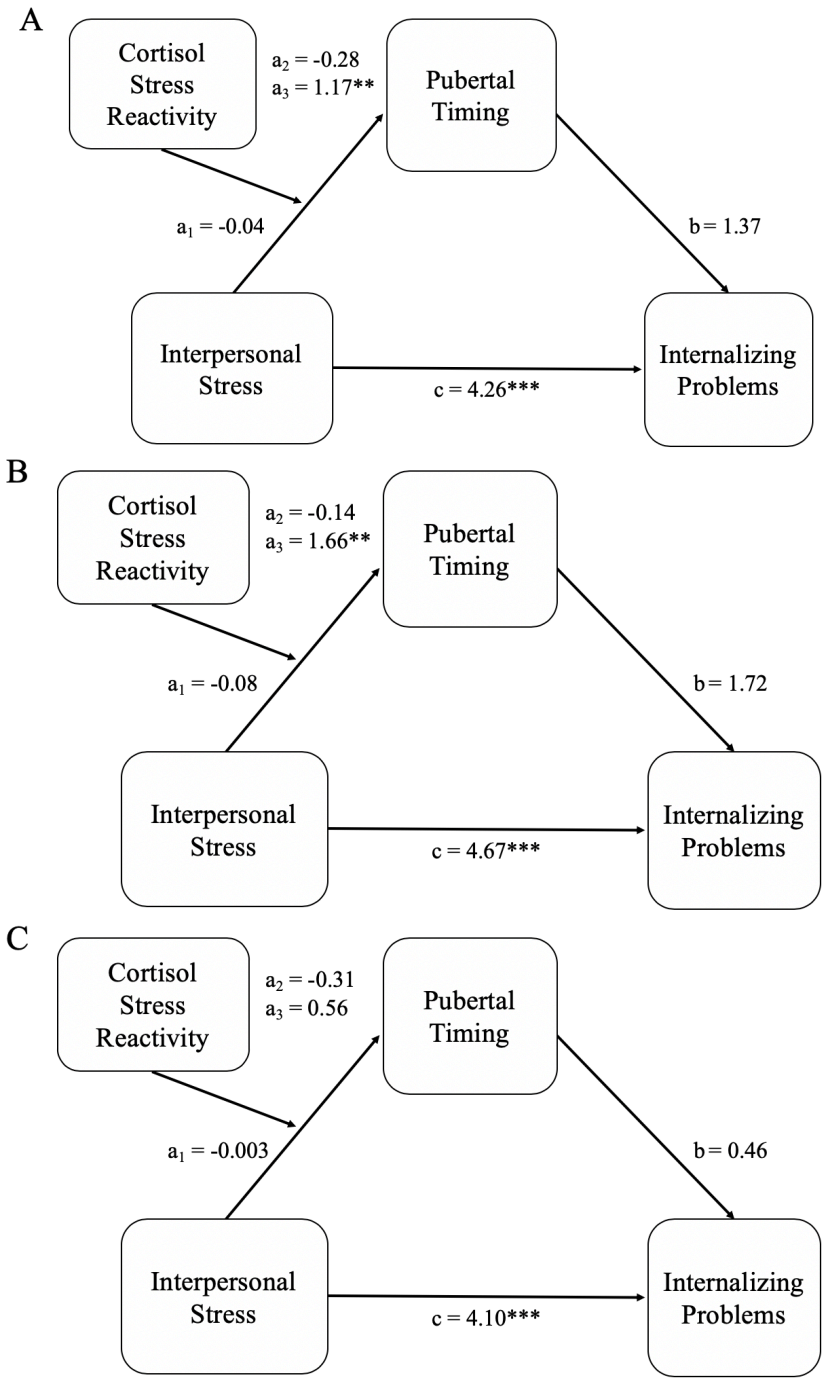
Study Assessment and Saliva Sampling Timeline



Note. Timings of saliva samples in relation to the assessment protocol are indicated by illustrations of salivettes. The first four salivettes are highlighted to show which saliva samples were included in assessment of cortisol stress reactivity.

Figure 3

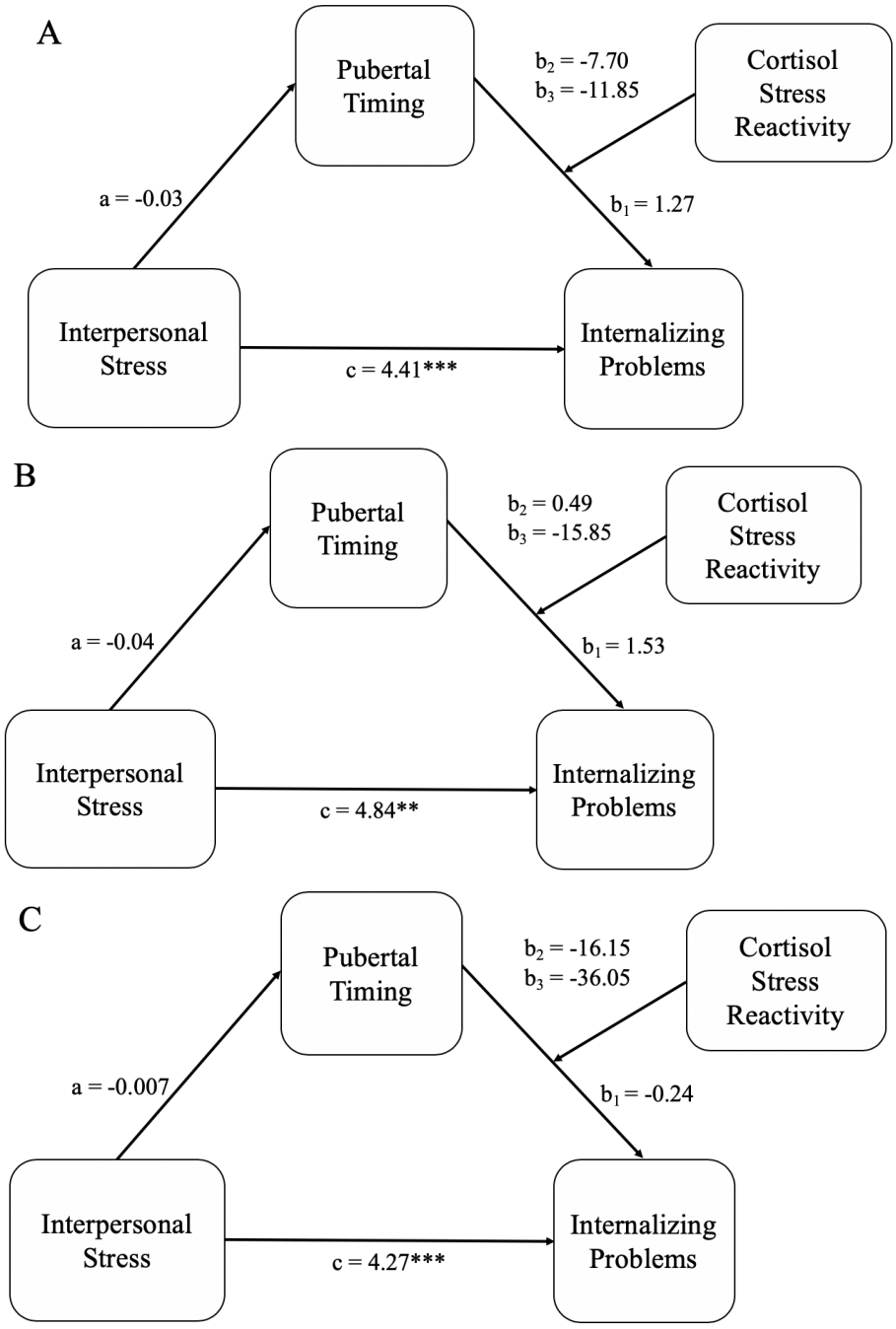
First-Stage Moderated Mediation Model of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems



Note. Panel A displays the model tested in the full sample, Panel B displays the model tested in females only, and Panel C displays the model tested in males only.
Unstandardized regression coefficients are reported.
a_2 represents the effect of cortisol stress reactivity on pubertal timing, and a_3 represents the effect of the interaction of stressful life events and cortisol stress reactivity on pubertal timing.
* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$

Figure 4

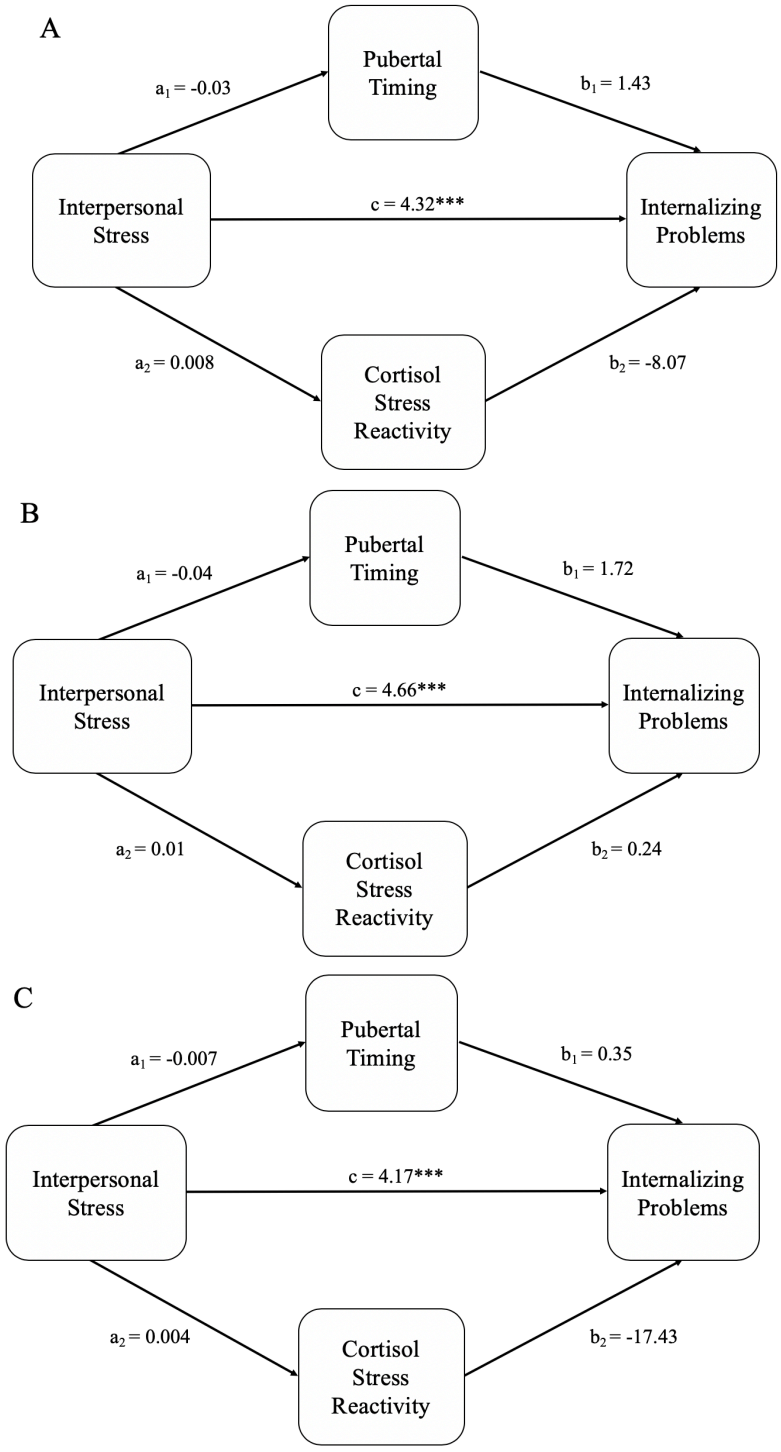
Second-Stage Moderated Mediation Model of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems



Note. Panel A displays the model tested in the full sample, Panel B displays the model tested in females only, and Panel C displays the model tested in males only.
Unstandardized regression coefficients are reported.
b_2 represents the effect of cortisol stress reactivity on internalizing problems, and b_3 represents the effect of the interaction of pubertal timing and cortisol stress reactivity on internalizing problems.
* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

Figure 5

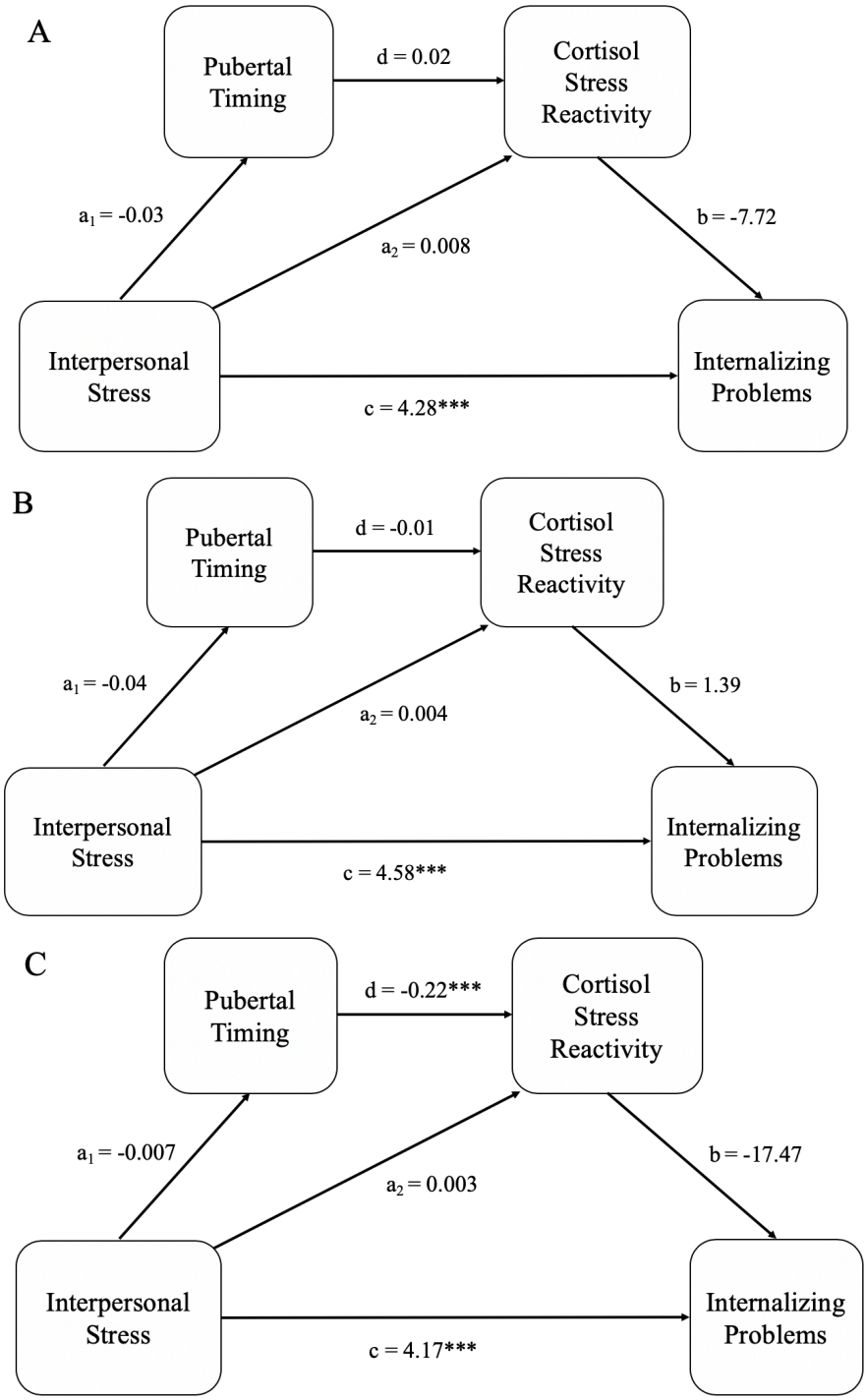
Multiple Mediation Model of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems



Note. Panel A displays the model tested in the full sample, Panel B displays the model tested in females only, and Panel C displays the model tested in males only.
Unstandardized regression coefficients are reported.
* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

Figure 6

Multiple-Step, Multiple Mediation Model of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems



Note. Panel A displays the model tested in the full sample, Panel B displays the model tested in females only, and Panel C displays the model tested in males only.
Unstandardized regression coefficients are reported.
* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

APPENDIX B

Table 1

Descriptive Statistics and Statistics Exploring Sex Differences in Study Variables

	Male	Female	Statistic
n	63	63	–
Age in years (range in years)	10.54 ± 0.83 (7.13–12.06)	10.54 ± 0.69 (8.95–11.76)	$t(124) = -0.03$
Race: Native American or Alaskan/Asian/White/Other (identified as Hispanic/Latino)	1/1/58/3 (3)	1/1/58/3 (1)	$\chi^2(3) = 0$ ($X^2(1) = 1.00$)
Annual household income (range)	$87,486 ± 78,147 ($13,639–500,000)	$133,344 ± 219,066 ($20,000–1,300,000)	$t(112) = -1.51$
Number of people in household (range)	4.44 ± 1.12 (2–7)	4.59 ± 1.20 (3–9)	$t(124) = -0.69$
Socioeconomic status (range)	$21,251 ± 22,138 ($4,667–133,333)	$25,184 ± 26,453 ($4000–125,000)	$t(112) = -0.87$
Internalizing problems t-score (range)	51.03 ± 11.12 (37–92)	54.21 ± 11.44 (31–88)	$t(124) = -1.58$
Number of interpersonal stress events endorsed (range)	1.22 ± 1.02 (0–4)	1.03 ± 1.18 (0–4)	$t(124) = 0.97$
Pubertal status (range)	1.44 ± 0.34 (1.00–2.25)	1.96 ± 0.59 (1.00–3.25)	$t(124) = -6.12$***
Pubertal timing (range)	0.00 ± 0.31 (-0.51–0.65)	0.00 ± 0.55 (-1.11–1.25)	$t(124) = -0.04$
Cortisol reactivity (range)	0.10 ± 0.08 (0.01–0.42)	0.10 ± 0.08 (0.00–0.39)	$t(124) = -0.33$

* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

Table 2

Correlations between Variables of Interest

	1	2	3	4	5	6	7	8
1. Internalizing problems		0.38**	0.00	-0.11	-0.02	-0.07	6.71*	-0.04
2. Number of interpersonal stress events endorsed	0.47***		-0.02	0.05	0.00	0.08	3.45	0.13
3. Pubertal timing	0.04	-0.09		-0.03	0.94***	0.04	0.71	-0.26*
4. Cortisol reactivity	0.09	0.17	0.08		-0.02	0.01	0.04	0.12
5. Pubertal status	0.04	-0.06	0.98***	0.12		0.37**	1.26	-0.25
6. Age	0.01	0.11	0.22	0.23	0.42***		0.95	-0.03
7. Medications	0.24	0.48	0.05	0.04	0.02	0.10		1.92
8. Socioeconomic status	-0.03	0.01	-0.09	-0.04	-0.07	0.06	0.07	

Note. Correlations above the diagonal are among males. Correlations below the diagonal are among females.
Zero-order correlations are reported for continuous variables. One-way ANOVAs are reported for medication differences.
Ns ranging from 53 to 63.
* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

Table 3

Hierarchical Multiple Regression Analysis of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems in Full Sample

	Model 1		*Model 2*		*Model 3*		*Model 4*	
	b	*SE*	*b*	*SE*	*b*	*SE*	*b*	*SE*
Intercept	51.03***	1.43	50.46***	1.31	50.46***	1.31	50.38***	1.32
Age[a]	-0.51	1.33	-1.09	1.22	-1.25	1.24	-1.26	1.25
Sex[b]	3.18	2.02	4.00	1.84	4.00*	1.85	4.08*	1.86
Interpersonal stressful life events	–	–	4.16**	1.28	4.17**	1.29	4.25**	1.29
Interpersonal stressful life events x Sex[b]	–	–	0.50	1.69	0.59	1.70	0.48	1.72
Pubertal Timing	–	–	–	–	0.59	4.18	0.48	4.19
Pubertal Timing x Sex[b]	–	–	–	–	1.50	4.83	1.58	4.85
Cortisol Stress Reactivity	–	–	–	–	–	–	-17.33	15.80
Cortisol Stress Reactivity x Sex[b]	–	–	–	–	–	–	19.84	23.57
R^2	.02		.20		.21		.22	

* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

[a]Age is mean-centered for full sample.

[b]0 = males, 1 = females.

Table 4

Hierarchical Multiple Regression Analysis of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems in Females Only

	Model 1		*Model 2*		*Model 3*		*Model 4*	
	b	*SE*	*b*	*SE*	*b*	*SE*	*b*	*SE*
Intercept	54.21***	1.45	54.46***	1.29	54.45***	1.30	54.45***	1.31
Age[a]	0.20	2.11	-0.65	1.88	-1.02	1.94	-1.07	2.00
Interpersonal stressful life events	–	–	4.63***	1.11	4.74***	1.12	4.72***	1.15
Pubertal Timing	–	–	–	–	2.02	2.46	2.00	2.48
Cortisol Stress Reactivity	–	–	–	–	–	–	2.16	17.70
R^2	.0001		.22		.23		.23	

* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

[a]Age is mean-centered for female sample.

Table 5

Hierarchical Multiple Regression Analysis of Effects of Interpersonal Stress, Pubertal Timing, and Cortisol Stress Reactivity on Internalizing Problems in Males Only

	Model 1		*Model 2*		*Model 3*		*Model 4*	
	b	*SE*	*b*	*SE*	*b*	*SE*	*b*	*SE*
Intercept	51.03***	1.41	50.46***	1.32	50.46***	1.33	50.38***	1.33
Age[a]	-1.01	1.72	-1.40	1.60	-1.41	1.62	-1.39	1.62
Interpersonal stressful life events	–	–	4.18**	1.30	4.18**	1.31	4.26**	1.31
Pubertal Timing	–	–	–	–	0.60	4.24	0.49	4.24
Cortisol Stress Reactivity	–	–	–	–	–	–	-17.32	15.96
R^2	.006		.15		.15		.17	

* indicates $p < .05$, ** indicates $p < .01$, and *** indicates $p < .001$.

[a]Age is mean-centered for male sample.