Puberty as a moderator on risk for psychopathology symptoms and autistic stereotypy exacerbation in adolescents with and without ASD

by

Jenny Mai Phan

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Human Development and Family Studies

Program of Study Committee:
Elizabeth A. Shirtcliff, Major Professor
Carla Peterson
Auriel Willette
Megan Gilligan
Amie Zarling

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2020

DEDICATION

I would like to dedicate this dissertation to my husband Bach and my mini team Levi, Matthew, Nicholas, and Olivia as well as my friends and family and extended family whose support has resulted in the completion of this research and Ph.D. program. I also dedicate this dissertation to all adolescents and their families who live with mental health challenges. More attention can be dedicated to reduce stigma around mental health disorders in society.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS

To my partner for life Bach who convinced me to give academia a try and see where it will take me, that initial and ongoing support has kept me going and soaring. I am at this milestone because of his unending love and support. My mini team Levi, Matthew, Nicholas, and Olivia are my inspirations, the reason for my endeavors, and my hope for a better future. My mom Thao (alloparent to my mini team) is the third reason for how I have made it through these years of study and work. To all my mini team's teachers, associates, daycare staff, principals, daycare directors, and respite workers, each and every person devoted their time, love, and partnership in this thing called community support. They have time and again demonstrated compassionate care and teaching, and to this as a result, I have peace of mind that my mini team are in good hands.

My mentor Dr. Elizabeth "Birdie" Shirtcliff, who took a chance taking in an underdog among a pool of qualified candidates to join her lab, has been the guiding voice and reason for the spark of this research journey and its continuation. Her support in the dual life of parenthood and graduate school has invited a culture of "can do both and succeed" with a lab that understands and supports one another.

I would also like to thank my coauthors as well as academic supporters Drs. Hill Goldsmith, Carol Van Hulle, Katherine Theall, Stacy Drury, Carla Mazefsky, Jennifer Pfeifer, Robert Laird, Connie Lamm, Paula Ruttle, and the Stress Physiology and Investigative Team. They all inspire me and have continually shown strong, positive support for my research interests. Finally, I would like to thank the National Institute of Mental Health for supporting this dissertation research through the training grant and to all of my committee members for their contributions to this dissertation.

NOMENCLATURE

O-A	Organizational-Activational Hypothesis
ASD	Autism Spectrum Disorder
WTP	Wisconsin Twin Project
NDAR	National Database on Autism Research
SCE	Social-Conscious Emotion Study
DHEA	Dehydroepiandrosterone
HPG	Hypothalamic-Pituitary-Gonadal Axis
HPA	Hypothalamic-Pituitary-Adrenal Axis

ABSTRACT

Mental health problems among adolescents remain a public health concern in the U.S. despite growing efforts to support mental wellbeing of youth. A part of the problem is a dearth of knowledge on evidence-based mechanisms on declining mental health in a particular subset of adolescents, and that subset is males and males on the autism spectrum. While studies on gender preponderance of mental health disorders contribute to the knowledge base on treating categorical psychopathology disorders sensitive to gendered issues, limitations include overlooking heterotypic comorbidities and understanding its underlying processes leading up to the development of mental health problems. Pubertal maturation is a process at which all adolescents transition through and for some adolescents, internalizing and externalizing symptoms may arise and can be overlooked. This is partly due to puberty viewed as a normative process for all adolescents going through "raging hormones," a misconception of the role of hormones and behavior during development. Along with the social and physical changes that come with adolescent development, neurobiological activities are taking place implicating brain development and behaviors. Hormones play a role in adolescent development; however, their mechanistic impact, particularly in males, is less understood.

This dissertation had three specific aims. The first aim was to investigate the effect of pubertal maturation on internalizing and externalizing (I-E) symptoms in children and adolescent males across development. The second aim was to examine the role of puberty and autistic stereotypy on I-E symptoms in typically developing and autistic youths. The third aim was to test the effect of pubertal hormone testosterone, physical changes, and autistic stereotypy on depressive symptoms in typically developing and autistic adolescent males. Findings from this

dissertation contribute to a small literature knowledge base on male adolescent development and psychopathology comorbidities.

CHAPTER 1. GENERAL INTRODUCTION

Puberty-related changes, coinciding with early adolescence, normatively impact emotion regulation, (Yazici, Bursalioglu, Aydin, & Yazici, 2013; Cohen, Tottenhan, & Casey, 2013; Maughan, Collishaw, & Stringaris, 2013) heightening risk for internalizing symptoms. Studies have largely focused on externalizing problems (e.g. conduct disorder) in males and internalizing symptoms in females (Angold & Worthman, 1993). This has led to female-focused psychosocial models of internalizing symptoms involving risk mechanisms (e.g., body image), which may not be directly applicable to males and risks ignoring the increasing number of males with internalizing symptoms (Hinshaw, 1992). Males still experience neurobiological and physical changes, and emotion regulation difficulties. These challenges may not be readily ascribed to puberty because hormonal and neural development in males is initiated long before physical maturation is visibly apparent to others (Schulz, Molenda-Figueira, & Sisk, 2009; Nelson, Leibenluft, McClure, & Pine, 2005). Neurobiological models that emphasize how puberty initiates a sensitive period in adolescent development (Hayward & Sanborn, 2002) by altering emotion-related neural networks may be applicable for males (Nelson, Leibenluft, McClure, & Pine, 2005; Guyer, Silk, & Nelson, 2016).

Adolescents with developmental disabilities such as Autism Spectrum Disorder (ASD) may be particularly susceptible to the emotion-regulation challenges that accompany puberty given that a hallmark characteristic of ASD is communication challenges and emotion dysregulation. With the unprecedented increase in ASD diagnoses in recent years (Boyle et al., 2011), it is imperative to fill in the knowledge gap regarding whether autistic stereotypy (defined as stereotyped behaviors of autism, such as repetitive movements or behaviors) is exacerbated by the experience of puberty, and to clarify the mechanisms by which puberty impacts autistic

stereotypies. Recent research has emerged on co-occurrence of internalizing symptoms and autistic stereotypy but needs further attention, particularly in boys. Given that the annual medical costs per year for children with ASD exceed those without ASD by four to six times (Perou et al., 2013) and with a higher prevalence of ASD among males (4.5 times more than girls), developing a better understanding of how autistic individuals navigate a vulnerable life stage may help address the rising comorbidity of mental health problems.

Another public health concern is that among adolescents ages 10 to 24 years, suicide is the third leading cause of mortality that account for over 4,600 deaths per year (81% of deaths among males and 19% among females; Center for Behavioral Health Statistics and Quality, 2016; Perou et al., 2013) and ultimately, reflect challenges with emotion regulation (Dahl, 2004; Sawyer et al., 2012). These emotion regulation challenges are associated with internalizing symptoms, such as anxiety and depression (Merikangas, He, Burstein, Swanson, Avenevoli, Cui, Benjet, Georgiades, & Swendsen, 2010; Angold, Erkanli, Silberg, Eaves, & Costello, 2002). Suicide is one illustrative example of the devastating effects of internalizing symptoms on individuals and families, helping to motivate this dissertation's focus on co-occurrence of internalizing symptoms in youth with ASD. While a comprehensive rate of suicidal ideation and attempts in youth on the autism spectrum is still unclear (Culpin et al., 2018; Chen et al., 2017; Hannon & Taylor, 2013), recent studies estimated a prevalence rate of suicidal behaviors to be between 7% and 42% in young people on the autism spectrum and found to be linked to depression and abuse (Hannon & Taylor, 2013). Emerging research has shown an increased association between youth with a diagnosis of autism and the likelihood of attempting suicide later in life (Chen et al., 2017; Veenstra-VanderWeele, 2018) and that suicidality was associated with social communication problems (Culpin et al., 2018). As a means to prevent suicidality in

the autism population, empirical work is needed to help elucidate processes that lead to suicidal behaviors, and that begins with risk factors, such as depression and anxiety left undetected and/or untreated. Much of these internalizing symptoms surface right around puberty (Greenlee, Mosley, Shui, Veenstra-VanderWeele, & Gotham, 2016; Eriksen, 2016), and there is still a dearth of studies tackling a better understanding of the mechanism of puberty in the autism population and co-occurring internalizing symptoms.

The goal of this research is to uncover how normative, yet hidden, challenges associated with puberty may impact emotion regulation challenges in autistic youth with co-occurring internalizing symptoms. The central hypothesis is that the pubertal transition, a sensitive period of development, may show manifestations of internalizing symptoms in youth and that autistic youth may be particularly susceptible due to known emotion regulation challenges (Picci & Scherf, 2015). This dissertation research leverages existing data across three complementary datasets, which contain information about puberty, internalizing symptoms and autistic stereotypy. The Wisconsin Twin Project (WTP) and the National Database on Autism Research (NDAR) has data on both typically developing and autistic adolescents that inform about autistic stereotypy and internalizing symptoms changes across adolescence and specifically, with reference to puberty. The Social Conscious Emotion study (SCE) permit examination of the association of puberty and symptoms in both typically developing and autistic adolescent boys. These are valuable data with information on internalizing symptoms and autistic stereotypy and a detailed panel of puberty measures, including hormones, which can address a critical need and directly relate to the aims of this study for understanding neurobiological processes in boys during puberty. There are 3 specific aims for this research:

Aim 1) To investigate the association between pubertal development and individual differences in internalizing and externalizing (for brevity, psychopathology) symptoms development in boys. This association was examined across the entire range of psychopathology symptoms in the WTP dataset across 3 waves. Subsequent analyses specifically compare groups typically developing males and males with elevated psychopathology symptoms. Longitudinal analyses were conducted to understand the influence of puberty and hormones. Findings from this aim will inform specific pubertal agents and psychopathology symptoms to better identify risk factors for future psychopathology.

Aim 2) To examine if pubertal development is associated with autistic stereotypy and internalizing symptoms in autistic males. Using the NDAR dataset, analyses were conducted to examine how puberty influences autistic stereotypy. Following a similar strategy as Aim 1 but cross-sectionally, subsequent analyses then compared internalizing and externalizing symptoms, as a function of pubertal development, between groups of typically developing and autistic males.

Aim 3) To explore neurobiological differences associated with puberty and autistic stereotypy. The SCE dataset was complementary to NDAR by allowing a deeper dive into the puberty-specific neurobiological differences between typically developing males and autistic males including the sex hormone testosterone. This aim was examined via regression analyses and was exploratory due to the dearth of studies on puberty in ASD.

Accomplishing these three aims shed light on typical and atypical developmental processes in adolescence, moving the field toward informing prevention and intervention strategies geared toward redirecting problem behaviors and symptoms toward healthy emotion regulation.

1.1 Significance and Rationale

The rationale for this research is that new research and models are needed to advance understanding of how early pubertal development may impact internalizing symptoms in males. This research used integrative approach combining psychopathology, neurobiological, and developmental disability models to examine pubertal transition in autistic adolescent males to inform prevention and treatment strategies.

1.2 Problem Statement

A critical gap in scientific knowledge concerns comorbidities between internalizing mental health and developmental disability symptoms in adolescence, focusing specifically on autistic stereotypy (e.g., rocking, flapping, scripting) and emotion regulation difficulties. This is an area of high importance due to its ability to inform translational public health research and improve care within these families. By examining how a universally experienced developmental transition such as puberty poses a substantial risk for certain youth, particularly symptom exacerbation of comorbidities between mental health and autistic stereotypy. Focusing the majority of research efforts on males, and males with autism in particular, will help fill a knowledge gap about a vulnerable understudied population across development. Examining the neurobiology underlying pubertal changes help to elucidate the mechanisms responsible for symptom changes and can inform future treatment and prevention efforts.

References

Angold, A., & Worthman, C. W. (1993). Puberty onset of gender differences in rates of depression: a developmental, epidemiologic and neuroendocrine perspective. *Journal of Affective Disorders*, *29*(2–3), 145–458. https://doi.org/https://doi.org/10.1016/0165-0327(93)90029-J

Boyle, C. A., Boulet, S., Schieve, L. A., Cohen, R. A., Blumberg, S. J., Yeargin-Allsopp, M., … Kogan, M. D. (2011). Trends in the Prevalence of Developmental Disabilities in US Children, 1997-2008. *Pediatrics*, *127*(6), 1034–1042. https://doi.org/10.1542/peds.2010-2989

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. *Annals of the New York Academy of Sciences*, *1021*(1). https://doi.org/10.1196/annuals.1308.001

Chen, M.-H., Pan, T.-L., Lan, W.-H., Hsu, J.-W., Huang, K.-L., Su, T.-P., … Bai, Y.-M. (2017). Risk of Suicide Attempts Among Adolescents and Young Adults With Autism Spectrum Disorder: A Nationwide Longitudinal Follow-Up Study. *The Journal of Clinical Psychiatry*, *78*(9), e1174–e1179. https://doi.org/10.4088/JCP.16m11100

Cohen, M. M., Tottenhan, N., & Casey, B. J. (2013). Translational developmental studies of stress on brain and behavior: Implications for adolescent mental health and illness? *Neuroscience*, *248*(26), 53–62. https://doi.org/https://doi.org/10.1016/j.neuroscience.2013.01.023

Culpin, I., Mars, B., Pearson, R. M., Golding, J., Heron, J., Bubak, I., … Rai, D. (2018). Autistic Traits and Suicidal Thoughts, Plans, and Self-Harm in Late Adolescence: Population-Based Cohort Study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *57*(5), 313-320.e6. https://doi.org/10.1016/J.JAAC.2018.01.023

Eriksen, W. (2016). Facing Puberty: Exploring The Onset, Symptoms And Experience Of Menses In Females With Autism Spectrum Disorder. *Publicly Accessible Penn Dissertations*. Retrieved from https://repository.upenn.edu/edissertations/2270

Greenlee, J. L., Mosley, A. S., Shui, A. M., Veenstra-VanderWeele, J., & Gotham, K. O. (2016). Medical and Behavioral Correlates of Depression History in Children and Adolescents With Autism Spectrum Disorder. *Pediatrics*, *137 Suppl 2*(Supplement 2), S105-14. https://doi.org/10.1542/peds.2015-2851I

Hannon, G., & Taylor, E. P. (2013). Suicidal behaviour in adolescents and young adults with ASD: Findings from a systematic review. *Clinical Psychology Review*, *33*(8), 1197–1204. https://doi.org/10.1016/J.CPR.2013.10.003

Hayward, C., & Sanborn, K. (2002). Puberty and the emergence of gender differences in psychopathology. *Journal of Adolescent Health*, *30*(4), 49–58. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11943575

Hinshaw, S. P. (1992). Externalizing behavior problems and academic underachievement in childhood and adolescence: causal relationships and underlying mechanisms. *Psychological Bulletin*, *111*(1), 127–155. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1539086

Guyer, A. E., Silk, J. S., & Nelson, E. E. (2016). The neurobiology of the emotional adolescent: From the inside out. *Neuroscience & Biobehavioral Reviews*, *70*, 74–85. https://doi.org/https://doi.org/10.1016/j.neubiorev.2016.07.037

Maughan, B., Collishaw, S., & Stringaris, A. (2013). Depression in Childhood and Adolescence. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *22*(1), 35–40. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3565713/

Nelson, E. E., Leibenluft, E., McClure, E. B., & Pine, D. S. (2005). The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology. *Psychological Medicine*, *35*(2), 163–174. https://doi.org/https://doi.org/10.1017/S0033291704003915

Perou, R., Bitsko, R. H., Blumberg, S. J., Pastor, P., Ghandour, R. M., G.C., J., … Huang, L. N. (2013). Mental Health Surveillance Among Children — United States, 2005–2011. *Morbidity and Mortality Weekly Report*, *62*(2), 2005–2011. Retrieved from https://www.cdc.gov/Mmwr/preview/mmwrhtml/su6202a1.htm

Picci, G., & Scherf, K. S. (2015). A Two-Hit Model of Autism: Adolescence as the Second Hit. *Clinical Psychological Science*, *3*(3), 349–371. https://doi.org/10.1177/2167702614540646

Sawyer, S. M., Afifi, R. A., Bearinger, L. H., Blakemore, S. J., Dick, B., Ezeh, A. C., & Patton, G. C. (2012). Adolescence: A foundation for future health. *The Lancet*, *379*(9826), 1630–1640. https://doi.org/10.1016/S0140-6736(12)60072-5

Schulz, K. M., Molenda-Figueira, H. A., & Sisk, C. L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior*, *55*(5), 597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

Veenstra-VanderWeele, J. (2018). Recognizing the Problem of Suicidality in Autism Spectrum Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*, *57*(5), 302–303. https://doi.org/10.1016/J.JAAC.2018.03.003

Yazici, E., Bursalioglu, F. S., Aydin, N. Y., & Yazici, A. B. (2013). Menarche, puberty and psychiatric disorders. *Gynecological Endocrinology*, *29*(12), 1055–1058. https://doi.org/doi: 10.3109/09513590.2013.829447

CHAPTER 2. LITERATURE REVIEW

This literature review provides an overview on developmental and biological factors that contribute to increasing internalizing problems in adolescents. This review begins with normative adolescent development and covers the topic on gender-differentiated pubertal development of secondary sexual characteristics and neurobiological processes that underlie these changes. Then, the next topic that is covered is on sex-preponderant psychopathologies during adolescence. Next, this review provides an overview on Autism Spectrum Disorder (ASD) and current national rates that represent diagnostic propensities in children. Lastly, the topic on neurobiological models that supports adolescence as a vulnerable developmental stage for autistic youth is covered.

One point to note that is reflected throughout this dissertation is nomenclature when describing the studied population. An ongoing debate between the use of person-first vs. identify-first language regarding the autism community (both lay and scientific communities) is acknowledged (Gernsbacher, 2017; Dunn & Andrews, 2015). By recommendation of the American Psychological Association (APA; American Psychological Association, 2020), person-first (individuals/person with autism/ASD) language and identity-first (autistic) language is used interchangeably throughout when describing individuals/population diagnosed with ASD or behaviors resembling autism. This suggestion from the APA was highlighted in the revised 7th edition Section 5.4 focusing on the disability population. The goal of Section 5.4 was for research writers to be informed about bias-free language that emphasizes inclusivity and respect of the studied population. Another nomenclature that is used throughout this dissertation is the use of “autistic behaviors” or “stereotypy” rather than symptoms in respect for those who view these phenotypes as a part of their identity and encompass a positive connotation when using the

quoted terms rather than as a part of an illness by use of the term “symptoms.” In the same thread, “symptoms” is used to describe internalizing problems (moodiness, irritability, depressive, and anxious behaviors), as well as used to describe externalizing problems (conduct disorder behaviors, oppositional defiant behaviors, aggressive behaviors). The term “symptoms” in this dissertation describes an undesirable manifestation of observed mental health outcomes. Therefore, to describe internalizing and externalizing outcomes, the term symptoms is used throughout this dissertation.

2.1 Gender and Puberty

2.1.1 Puberty Initiates Normative Developmental Stage of Adolescence

Adolescence is a transitional developmental stage that begins with the onset of puberty and ends with the attainment of adult roles and responsibilities (Blakemore, Burnett, & Dahl, 2010). All children, regardless of neurodevelopmental status, experience rapid anatomical growth, abrupt physiological and psychological alterations, and social identity changes. Many normative developmental changes are initiated by pubertal hormones (Shirtcliff, Dahl, & Pollak, 2009). Along with the development of secondary sexual characteristics, pubertal hormones alter neurotransmitter systems that impact emotion and mental health symptomologies, including internalizing symptoms (Rubinow & Schmidt, 1996; Simerly, Swanson, Chang, & Muramatsu, 1990). Hormonal regulation and dysregulation is not new in adolescence (Forbes & Dahl, 2010) but culminates from organizational, neurobiological effects, and environmental interactions that shape children’s development beginning *in utero* and for the first years of life (Schulz et al., 2009). Childhood hormones also shape emotional wellbeing (Martel, Klump, Nigg, Breedlove, & Sisk, 2009). In sum, pubertal maturation is a normative process initiated early in development, shaped by environmental forces, and then activated and re-organized across adolescence as a culmination of prior developmental processes.

2.1.2 Sex Comparison in Neurobiological Pathways and Characteristics of Puberty

A process that occurs before puberty called adrenarche begins around six to eight years of age in girls and seven to nine years of age in boys (Dorn, 2006; Blakemore et al., 2010), though there are individual differences in timing of adrenarche (Byrne et al., 2017; Marceau, Ram, Houts, Grimm, & Susman, 2011). Adrenarche is largely driven by dehydroepiandrosterone (DHEA) and DHEA-S (the sulfate form of DHEA). These steroid hormones are byproducts of the hypothalamic-pituitary-adrenal (HPA) axis, which are released from the adrenal cortex into the bloodstream following a cascade of hormones: corticotropin-releasing hormone (CRH) from the hypothalamus to adrenocorticotropic hormone (ACTH) from the pituitary gland (Louis et al., 2008). Pre-pubertal children during adrenarche would show elevated levels of DHEA, DHEA-S, and androstenedione, which are associated with physical changes, such as oily skin and body odor (Auchus & Rainey, 2004). DHEA and DHEA-S levels continue to stay elevated into adulthood (Orentreich, Brind, Rizer, & Vogelman, 1984) and has a diurnal and seasonal rhythm (Matchock, Dorn, & Susman, 2007). Much of these underlying and physical changes occur with minimal consequential effects on child development and behavior mostly due to it being less noticeable or visible than the changes that follow when puberty ushers the child into adolescence. Puberty begins with pubarche (first appearance of pubic hair) and thelarche (onset of breast development in girls) as well as gonadarde (testes and penis enlargement in boys) when changes become noticeable.

The mark of pubarche and thelarche in adolescents is associated with the activation of the hypothalamic-pituitary-gonadal (HPG) axis. The HPG system releases floods of a panel of hormones that spark both neural (invisible) and secondary sexual characteristic (visible) changes. This process is called gonadarche. In this panel of hormones, there are precursor hormones or gonadotropins, namely, luteinizing hormone (LH) and follicle stimulating hormone (FSH) that

are released from the pituitary gland prior to gonadal activation. This result in gonadal steroids, specifically testosterone and estrogen deriving from the testes and ovaries (respectively), releasing into blood circulation (Blakemore et al., 2010; Romeo, 2003). Gonadotropins (LH and FSH) are activated by the release of gonadotropin-releasing hormone (GnRH) in the hypothalamus via GnRH neurons (Sisk & Foster, 2004; Herbison, 2016). GnRH is directly linked to pubertal maturation of gonadal activity and is sensitive (specifically) to the negative feedback mechanism of testosterone and estrogen released from the gonads. Pubertal onset or timing has been theorized to be associated with the GnRH system and its sensitivity to gonadal steroids' negative feedback control in which may also be linked to prenatal gonadal steroid levels in some individuals (Sisk & Foster, 2004). The organizational-activational hypothesis (O-A) fits with this understanding, and its theory is that prenatal gonadal steroids *in utero* are associated with postnatal steroid hormone levels during adolescence, where these steroid hormones influence reproductive behaviors at around puberty (Schulz et al., 2009; Wallen, 2009; Phoenix, Goy, Gerall, & Young, 1959). To put in another way, neural networks organize and work in conjunction with the gonadal system *in utero* and during the neonatal period (Morris, Jordan, & Breedlove, 2004); then later during adolescence, the same neural and gonadal systems are activated, which starts puberty. Emerging research have theorized that the O-A might apply before puberty (i.e., adrenarche) in which the sensitive period of development has a wider window than previously thought to be exclusive to gonadarche (Byrne et al., 2017). Figure 1 illustrates the processes of pre-pubertal to pubertal changes in females and males.

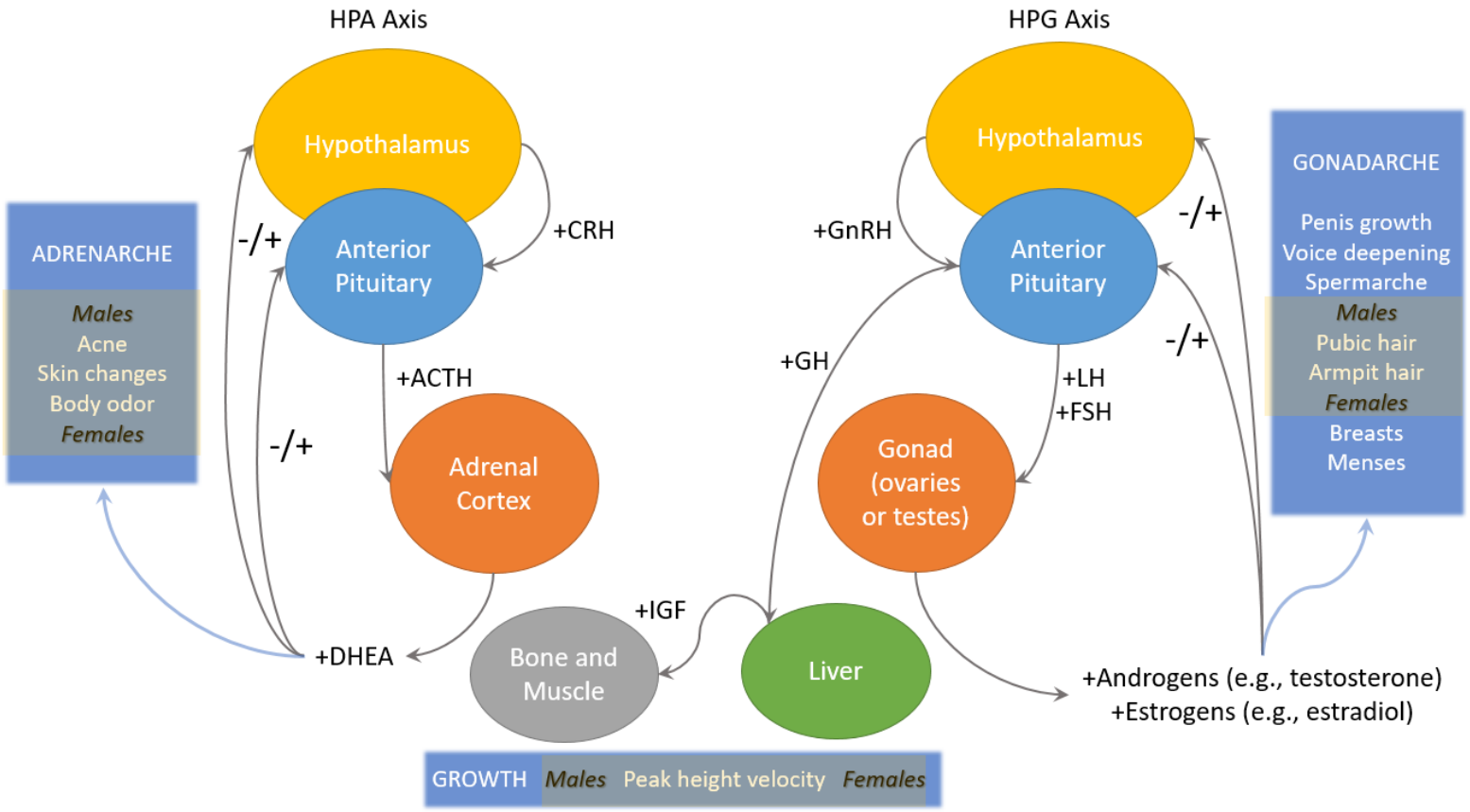

Figure 2.1 *Hormonal byproducts of the HPA-HPG axes during adrenarche and gonadarche*

Neuronal cells from the hypothalamus send signals to the anterior pituitary gland to release intermediary hormones (CRH, ACTH, GnRH, LH, FSH) to target endocrine and gonadal glands. Pubertal hormones (e.g., DHEA, testosterone, estradiol) are then released due to positive feedback mechanism. During slower pubertal maturation, negative feedback mechanism then causes decreasing release of intermediary hormones (-/+), which diminishes pubertal hormones. During increasing pubertal maturation, positive feedback mechanism continues pubertal hormones release in the bloodstream (-/+). CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; GnRH, gonadotropin-releasing hormone; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IGF, insulin growth factor (adapted from Louis et al., 2008 *Pediatrics; vol 121)*

2.1.2.1 Puberty for females

For human females, secondary sexual characteristics become visible by the first sign of pubic hair growth and then breast development. Pubic hair and breast development are broken into five stages called Tanner Stages (see complete review in Marshall & Tanner, 1969). Visible signs include skin coloration, breast size, areola diameter, hair growth and quantity, and hair texture. Another sign of puberty for girls is starting her first period or menstrual bleeding called menarche. About 90% of females begin menarche even before breast development has reached its fully matured stage (Marshall & Tanner, 1969; Cabrera, Bright, Frane, Blethen, & Lee, 2014). In general, mean age of menarche is around 12 years of age. When examining race/ethnic differences in age of menarche in the U.S., no significant differences were found; menarche on average begins at around 12 years of age for Non-Hispanic, Caucasian, for Black, African American, for Asians, Asian Americans or Pacific Islanders, and at around 11 years of age for Hispanic, Latina females (Cabrera et al., 2014; Biro et al., 2018). From beginning of breast development to menarche, breast development tempo (or rate of change over time) is on average 2.3 years with a range of 6 months to 5.75 years (Marshall & Tanner, 1969; Marceau, Ram, Houts, Grimm, & Susman, 2011).

Pubertal maturation in girls is driven by the gonadal steroid estrogen (specifically, estradiol) and progesterone. Estradiol has been found to have direct positive feedback on GnRH neurons (Herbison, 2008; Herbison, 2016), which leads to reproductive development and ovulation during the menstrual cycle. During the menstrual cycle, estradiol typically peaks at around ovulation, and during menstruation, estradiol has lower levels than at ovulation and a non-linear trajectory while progesterone has a curvilinear trajectory (Häggström, 2014). Females have more visible changes during puberty than males, and therefore, generally, females can

receive more support and care as they undergo these changes. However, some females might not receive proper support when pubertal changes are stigmatizing (Winkler & Roaf, 2015).

2.1.2.2 Puberty for males

While for girls, most visible signs of puberty begin at Tanner stage 2. Many of the first signs of puberty in males are "hidden." For example, it is not until males are Tanner stage 4 (on average) that they experience a growth spurt and develop facial hair, which are hallmark visible signs of male maturation (see Marshall & Tanner, 1970 for review). Prior to these visible changes, males experience similar pubertal changes as females, and these changes are associated with adrenal development also called adrenarche. Adrenarche starts a chain of pubertal events, which includes acne, oily skin, and body odor. Shifting from the HPA axis (adrenarche) to the HPG axis (gonadarche) and its role on secondary characteristic changes during puberty, males begin to show pubic hair, testicular, and penis growth reach peak growth velocity at around Tanner stage 3 to 4 (Bordini & Rosenfield, 2011; Zawatski & Lee, 2013). Voice deepening tends to begin at around Tanner stage 3 and permanent adult-like voice set in at around Tanner stages 4 to 5. Peak height velocity begins at around Tanner stage 3 and ends around Tanner stage 5. Although depending on when growth spurt begins, Tanner staging for peak height velocity varies by timing and tempo of height growth driven by total output of pubertal hormones (e.g., insulin-like growth factors, growth hormones, estrogens, androgens, and thyroid hormones; Rogol, 1992). Another developmental milestone during gonadarche for males is the first sign of spermarche, an occurrence during mid-puberty when males develop mature sperm cells in the testes, that is released through the mechanism of gonadotropins and testosterone (Zawatski & Lee, 2013). Males entering gonadarche are as early as age ten years for Non-Hispanic, Caucasian

and Hispanic, Latino youth and at around age nine for Black, African American males (Herman-Giddens, Wang, & Koch, 2001).

The HPG axis functions similarly in males as females, except that in males the GnRH pulse generator (pulsatile secretions of GnRH) activates gonadotropins to stimulate Leydig cells in the testes and to ultimately release testosterone into the bloodstream. As mentioned above, testosterone (as well as estrogen and progesterone) has a negative feedback mechanism to maintain homeostasis of hormone circulation in the body; however, there is also a positive feedback mechanism between gonadotropins (GnRH, FSH, LH) that plays an important role in testosterone (as well as estrogen) regulation (Foster, Jackson, & Padmanabhan, 2006). The intricacy of hormones feedback mechanism is one key in understanding the longitudinal process of the O-A and its ecological interaction and impact on adolescent health and wellbeing. Pubertal hormones are important mediators that informs how the human body and its genetic correlates interact with the environment, which explain phenotypic outcomes (e.g., behaviors, traits, psychiatric conditions). These mechanisms can provide evidence for a path leading to mental health symptoms in some youth, and therefore, it is worthwhile to further examine puberty and pubertal hormones in vulnerable as well as understudied populations.

2.2 Gender and Psychopathology

2.2.1 Adolescence Is A Vulnerable Life Stage for Internalizing Symptoms

According to the National Survey on Drug Use and Health (Center for Behavioral Health Statistics and Quality, 2016), 12.5% of the U.S. population ages 12 to 17 years experienced at least one episode of major depression (Center for Behavioral Health Statistics and Quality, 2016) and 3% experienced anxiety (Perou et al., 2013). Given the insidious nature of internalizing problems, signs and symptoms can go unnoticed when adolescents mask or hide these symptoms

(Kokkevi, Arapaki, & Richardson, 2011), or when adolescents struggle to articulate emotional challenges. Rates of depression and anxiety in adolescents have increased since 2007, with females between ages 10 and 14 years showing a rise of 13% and males a rise of 7% (Ruch et al., 2019). Given that research supports the notion of heterotypic comorbidity, in which one disorder predicts the development of another discrete disorder, advancing understanding of internalizing disorders can also help mitigate the increase of problem behaviors observed in males later in adolescence (Mazefsky et al., 2013; Samson et al., 2014; Costello, Erkanli, & Angold, 2006).

In understanding psychopathology in children and adolescents and its manifestation in complex ways, first, there are two principles to discuss: equifinality and multifinality (Cicchetti & Rogosch, 1996). In the context of psychopathology in youth, equifinality refers to youth developing *similar* psychopathologies resulting from *different* developmental paths. The multifinality principle holds that youth with *similar* developmental paths result in *different* psychopathology outcomes. These principles would be best explained by those developmental and psychopathology factors that contribute to those pathways. This literature review, however, focuses on factors that are most relevant to preponderant mental health disorders during puberty, in adolescence, and based on gender.

The application of the equifinality principle lies in, for instance, children with developmental disorders (e.g., autism, attention-deficit-hyperactivity disorder, language disorders) who had different upbringings, therapy treatments, and genetic and environmental correlates that explained their development of depression and anxiety. In order to describe the applicability of these two principles, I provide two vignettes. In the case of Autistic Youth One undergoing puberty, this youth experienced a neglectful upbringing, did not receive therapy treatments, and did not have the SCN2a candidate gene (commonly found in autism samples but

not in all cases; Freed & Pevsner, 2016) developed depression and anxiety disorders. In another case, a different Autistic Youth Two had a supportive upbringing, received therapy treatments, and had the SCN2a candidate gene, and yet, this youth developed depression and anxiety disorders – *different* developmental pathways leading to a *similar* psychopathology outcome. Potential research questions relevant to this review that might help explain this outcome deriving from two different developmental pathways: 1) Was the youth male or female? 2) Was their adolescent experience positive or negative, regarding intrinsic controls and social interactions? 3) Was pubertal development a predictor of their depression and anxiety? The same example can be applied using the multifinality principle. Borrowing the description of the Autistic Youth Two and in a third vignette of two different youth, these two individuals are diagnosed with different mental health disorders: one with a stress disorder and the other with depression – *similar* developmental paths leading to *different* psychopathology outcomes. The same research questions can apply to this third vignette. Was the youth male or female? Was their adolescent experience positive or negative? Was pubertal development a predictor of their psychopathologies? Each of these examples are too simplistic to capture the full picture of pathways leading to mental health outcomes but provide an idea of how to carefully examine developmental models that combine psychopathology outcomes.

Zahn-Waxler, Shirtcliff, and Marceau (2008) conducted a review on gender and psychopathologies during child-onset and adolescent-onset mental health disorders. Extant literature supported a female preponderance of internalizing disorders, such as depression, anxiety, and eating disorders commonly appear during adolescent years, especially for females at risk for internalizing disorders. Contrarily, for males, who are at risk for internalizing disorders, these tend to show up more during childhood years, particularly around adrenarche (Angold et

al., 1996). Despite onset in adolescence, there is heightened risk for internalizing problems to persist across adolescence into adulthood (Steinberg, 2004). Previous studies that found this link usually examine gender differences rather than focus on within group analysis (e.g., males only). These findings leave out a subset of males who experience a worsening of internalizing symptoms during adolescence. Supported by extant literature, male-preponderant disorders lie in the externalizing domain (e.g,, attentional deficit hyperactivity disorder, autism spectrum disorder, conduct disorder, oppositional defiant disorder) and during child-onset (Zahn-Waxler et al., 2008; Zahn-Waxler, Crick, Shirtcliff, & Woods, 2006). While adolescent-onset of internalizing disorders are more common in females, a subset of males still struggles with depression and anxiety, and symptomologies of internalizing disorders as well as signs leading up to the disorder may present differently in males than they do in females. Given that studies show gender-differentiated disorders when comparing between males and females, within-gender variance of risk symptoms (mood, affect, forms of aggression, rumination) is worth further examining to test how the construct of particular behaviors and traits appear differently based on gender differences and how they manifest in comparison that ultimately lead to similar outcomes, such as feelings of worthlessness, extreme guilt, and suicidal ideation (Zahn-Waxler, Crick, Shirtcliff, & Woods, 2015). It is important in research to gain understanding in who is at risk for internalizing disorders and when children are most vulnerable in developing internalizing problems, and this begins by examining pathways of symptoms mapping onto environmental and biological factors.

2.2.2 Puberty Shapes Internalizing Symptoms in Males and Females Differently

For many adolescents, factors that play key roles in escalating challenging problems exist before they reach puberty, and this developmental transition can exacerbate these underlying

problems for those already at risk. Research is needed to best identify the period at which adolescents are most vulnerable and showing signs of worsening problems. Pubertal development is linked with increased mental health problems, particularly in those with early onset of internalizing symptoms (Hayward & Sanborn, 2002; Zahn-Waxler, Shirtcliff, & Marceau, 2008). Risks associated with puberty in males remain underappreciated because psychosocial models developed for females are applied to males' development, despite the fact that the underlying risk mechanism for puberty developed for females is unlikely to be applicable for males (Deardorff, Hoyt, Carter, & Shirtcliff, 2019). Whereas psychosocial mechanisms (e.g., body type and image) are unlikely to carry risk for males as it does for females, males may still experience adjustment challenges to a new neurobiological and physical form. Emotion regulation difficulties instantiated at a neurobiological level may have already been present for years given that males' hormonal and neural puberty begins long before external physical maturation is apparent to others.

Changes in internalizing symptoms might not be ascribed to puberty or pubertal hormones; instead, parents and practitioners may struggle to understand and help males through this developmental transition. Pubertal maturation is important for both genders, yet new insights are needed to understand how internalizing risk unfolds. Understanding processes that explain internalizing symptomologies and protective factors that buffer internalizing symptom increases will help inform prevention or intervention efforts that address this important public health concern.

2.2.3 Co-occurrence of Externalizing Symptoms in Males during Puberty

While it is important to focus on internalizing symptoms in males largely due to gender-related mechanisms during puberty are still unknown and less frequently studied than in females

(Mendle & Ferrero, 2012), it is hard to overlook externalizing symptoms that may overlap or co-occur in males. Externalizing behaviors can be characterized by aggression, risk-taking, oppositional defiance, hostility, conduct problems, antisocial, and attention deficits (Walton, Ormel, & Krueger, 2011; Lahey et al., 2008). Adolescents globally engage in more externalizing behaviors, particularly in risking-taking behaviors, such as experimenting with drugs, sex, and rule-breaking, simply to name a few, and these behaviors are found in both male and female adolescents (Negriff, Fung, & Trickett, 2008; Mendle & Ferrero, 2012). Increase in externalizing behaviors during adolescence has been linked to pubertal timing in both early and late maturers (Negriff et al., 2008). Externalizing behaviors during puberty may appear a normative developmental process in males and females; however, the co-occurrence of internalizing symptoms (e.g., depression) is less normative and deserves further investigation where it is most understudied (i.e., in males). More awareness on heterotypic comorbidity of internalizing and externalizing symptoms are making way in research to tap into more transdiagnostic measures (or a diagnosis-agnostic approach; Kushki et al., 2019) to best capture etiology of adolescent mental health problems. Methodologies like factor analyses are useful for determining symptoms that load onto separate domains of internalizing and externalizing disorders and when overlaps occur (Lahey et al., 2008). In other words, a symptom-level approach rather than a categorical measure that separates each psychopathology domain can be more informative to study gender-related models of puberty and adolescent psychopathology (Carragher et al., 2016).

Emerging research suggests taking the transdiagnostic approach when studying internalizing and externalizing model of psychopathology. One longitudinal study examined rumination behaviors in adolescents who exhibited aggressive behaviors and further explored gender differences in this transdiagnostic approach (McLaughlin, Aldao, Wisco, & Hilt, 2014).

In their study, researchers found rumination behaviors to mediate the association between depression and anxiety symptoms with subsequent aggressive behaviors over time spanning over 7 months in males, which indicated rumination to be a transdiagnostic factor bridging internalizing and externalizing psychopathologies in males. An extension of this transdiagnostic approach include a dimensional model of autistic behaviors or stereotypy (Rodriguez-Seijas et al., 2019; Noordhof, Krueger, Ormel, Oldehinkel, & Hartman, 2015). Within the Tracking Adolescents' Individual Lives Survey (TRAILS) study, Noordhof and colleagues (2015) found integrating autistic stereotypy with internalizing and externalizing symptoms using factor analysis to be useful in determining up to four factors of psychopathology: internalizing, externalizing, autism, and attention and orientation. Additionally, their study also found a bi-factor of psychopathology that can have utility for clinical practice, which is important for intervention work within the autism population

Another transdiagnostic methodology that can be useful is examining symptoms severity and directionality of symptoms. Essex and colleagues (2006) suggested using a mathematical calculation to capture comorbidity by computing a severity score — strength of internalizing or externalizing symptoms — and a directionality score —symptoms higher in the internalizing or externalizing domain. The calculation is simply: (externalizing + internalizing / 2) = severity, and (externalizing – internalizing) / 2) = directionality. A different but similar strategy in extracting severity and directionality scores is by principal component analysis (PCA). A PCA is useful when there are multiple informants on psychopathology symptoms (Kraemer et al., 2003; Shirtcliff & Essex, 2008). Component loadings when fixed to two factors should load one factor for severity and the second factor for directionality. Extraction of factor scores from the PCA would have scores (for severity) that show a standardized scale for level of symptoms and factor

scores with a positive or negative signifier (directionality) indicating preponderance of psychopathology domain. These transdiagnostic methodological approaches all serve the purpose of acknowledging there are comorbidity of psychopathologies in some adolescents. These analysis strategies account for some more global view of psychopathology and take into consideration individual differences that lead to mental health outcomes.

2.3 Autism Spectrum Disorder

2.3.1 Developmental Milestones and Diagnosis

Currently, in the United States, 1 in 59 children are diagnosed with ASD and on average at 8 years of age (Baio et al., 2018). Caregivers generally notice signs and symptoms that deviate from typical developmental milestones in children around 2 years of age and as young as 12 months of age. Autism is a neurodevelopmental disorder that includes all race and ethnic backgrounds, genders, socioeconomic status, and educational background. A stronger prevalence in Filipino- and Vietnamese-born children in the U.S. (Becerra et al., 2014), in males, and lower socioeconomic status (see Baio et al., 2018; Perou et al., 2013; Durkin et al., 2017) have been reported. As more awareness are spreading across the U.S., more people are learning signs of autism and recognize ASD earlier in development. As increased awareness reaches across the United States, the number of children diagnosed with ASD becomes even more prevalent. ASD was once termed mental retardation (Schalock, Luckasson, & Shogren, 2009) and was categorized as Pervasive Developmental Disorder, Childhood Disintegrative Disorder, Rhett's Disorder, Asperger's Disorder, and Autistic Disorder in the DSM-IV (American Psychiatric Association, 1994). Today, more commonly and accepted categorization of autism is developmental disability, and many know of the DSM-5 diagnostic label as Autism Spectrum Disorder with severity levels ranging from mild to moderate to severe types (American

Psychiatric Association, 2013). Though functioning labels, such as “high- vs. low-functioning,” are still widely used and fit with the previous labeling from the DSM-IV, severity types (mild, moderate, severe) are generally more accepted labels in the autism community and consistent with the DSM-5. Some autism communities continue to use severity levels as in previous DSM versions ranging from 1-3 with 3 as strongest in severity. The new direction in diagnostic labeling of ASD is to emphasize that autism has a range or spectrum of behaviors and traits that overlaps even among severity types, which also contributes to the prevalence rates due to the expansion of the spectrum. Due to the increased awareness, more children are evaluated today and subsequently receive an ASD diagnosis at three years of age (Mandell, Novak, & Zubritsky, 2005). Some of the common traits detected in toddlers with ASD are repetitive behaviors, deficits in verbal and nonverbal communication, erratic or irritable behaviors, social responsiveness deficits, and avoidance of eye contact.

2.3.2 Neurodevelopment of Autism

Research on prognosis of ASD over time are inconsistent and difficult to decipher due to the complexity and variability of autism across individuals. The utilization of screening tools and evaluation process with healthcare professionals (e.g., psychiatrists, clinical psychologists, neuropsychologists, developmental pediatricians) to detect signs of autism early in development prior to age one has been recommended so that early intervention can also begin. Dawson and colleagues (2012, 2005) conducted a series of studies examining children’s brain development and neuronal activity among control samples and children ‘at-risk’ for ASD (siblings). The purpose of these studies was to detect neuroanatomy and activity differences within the brain that can predict a later diagnosis of autism. Much of these children as early as age one who showed larger head circumference (Courchesne, Carper, & Akshoomoff, 2003), increased tissue white

matter (Courchesne et al., 2001), and vast amount of neuron firing (Belmonte et al., 2004) later showed significant developmental delays and traits indicative of autism. Head circumference overgrowth generally occurs postnatally within the first six to fourteen months, coinciding with what is normally a period of synaptogenesis, arborization of dendrites, and ongoing myelination (Courchesne, 2004). Regionally, frontal lobes show the greatest degree of enlargement and occipital lobes the least. Abnormalities are more pronounced in the frontal and temporal lobes. These are regions that mediate the higher-order social, emotional, cognitive, and language functions and are differentiated in autism. One reasonable explanation for early overgrowth in autism is an excess of neuron numbers, particularly an excess of excitatory pyramidal neurons (excess of axons, dendrites, synapses, and myelin), which produce the enlarged volumes of gray and white matter and overall enlarged brain volume reported in Magnetic Resonance Imaging (MRI) studies of young autistic children. Excess neurons could also mean increased brain weight.

Autism is a complex neurodevelopmental disorder that displays many idiosyncratic behaviors compared to typically developing individuals, and research has found different brain morphometry and neurocircuitry that are associated with those behaviors unique to those with ASD. However, consistent findings in neurobiological and behavioral correlates have been an issue in the autism research literature. This may be partly due to two problems in research: 1) aggregating mild to moderate to severe types and drawing interpretation of findings as one ASD type and 2) replication of study designs but with different severity type (inconsistent with previous studies). While such neurobiological correlates of autistic behaviors align with diagnostic measures, especially in early development, it is still unclear as to the level of variability in these associations in older autistic individuals.

I propose a developmental biopsychosocial model guided by the two-hit autism model (Picci & Scherf, 2015) that encompasses puberty as a risk factor for increasing internalizing symptoms in autistic adolescent males. This approach will exert a broader impact by leading to improvements in scientific knowledge and provide useful information for prevention and intervention policies.

2.4 Two-Hit Autism Model

2.4.1 Autistic Youth Undergo Two-Hit Changes During Puberty

Youth with developmental disabilities such as autism may face additional barriers to communication of emotion regulation-based problems. Autism is typically diagnosed in childhood, with greater prevalence for males, yet they do not "grow out" of autism with age (Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). The two-hit model of autism (Picci & Scherf, 2015) postulates that autistic youth go through early neural perturbations as a *first-hit* and then neural reorganizations during puberty as a *second-hit*. The two-hit model suggests that adolescence can be a confusing period for autistic youth as neurobiological changes (due in part to puberty) can heighten emotion regulation difficulties and autistic stereotypy exacerbation (also called stimming). Communication problems add to the constraints that autistic youth already face (Samson et al., 2014), which may heighten irritability and sensitivity. Normative emotion regulation challenges of adolescence are also experienced by autistic youth and, for some, will manifest as increased internalizing symptoms. Without the means to articulate their mental health concerns and without targeted treatment for internalizing symptoms designed specifically for autistic youth, the normative challenges of autistic adolescents can spiral rapidly and ultimately lead to problems that overwhelm their psychological capacity to self-regulate (Picci & Scherf, 2015). Unfortunately, developmental models for comorbidities are based on theories of

neurotypical youth or youth with mental health problems (Chiri & Warfield, 2012; Weiss et al., 2018) and extrapolated to autistic adolescents, or conversely, developmental models are developed for autistic children and applied across the lifespan without fully incorporating the new biopsychosocial challenges of puberty and adolescent development. Empirical research is needed on autistic youth as they develop from childhood, across adolescence, and emerge into adulthood.

2.4.2 Co-occurrence of Autistic Stereotypy and Psychopathology Symptoms

It is important for research on autism to tie in processes that predict adjustment problem trajectories in autistic adolescents. This knowledge gap is especially problematic for understanding the processes of autistic stereotypy across development because autistic adolescents have added difficulties, which include (a) puberty directly impacts autistic stereotypy as it becomes difficult for youth to adjust during puberty, and (b) underlying autistic stereotypy become exacerbated by new challenges to emotion-regulation and internalizing comorbidities. Furthermore, studies need to parse out internalizing symptoms from autistic behaviors and vice versa to better determine how pubertal hormones and the pubertal process really impact youth, especially in males.

A dearth of empirical studies have focused on predictors of emotion dysregulation in autistic adolescents, while a few studies found a list of predictors of emotion dysregulation in young children on the autism spectrum, which includes autistic stereotypy severity and executive functioning ability (Cibralic, Kohlhoff, Wallace, McMahon, & Eapen, 2019) as well as cognitive and language abilities (Berkovits, Eisenhower, & Blacher, 2017) and social skills (Neuhaus, Webb, & Bernier, 2019). Emotion dysregulation has several labels and definitions (e.g., affective instability; Marwaha et al., 2014); and therefore, interpretations of emotion regulation measures

(including neurobiological and behavioral; Broome, He, Iftikhar, Eyden, & Marwaha, 2015) and their association with a measured outcome can vary and may produce low generalizability, unless clearly defined within a standardized empirical construct. As mentioned above in Section 2.2.3, a transdiagnostic approach that is data-driven to determine factors contributing to mental health problems in autistic adolescents would better serve the autism community due to a high co-occurrence of other psychopathologies.

2.5 Research Questions and Hypotheses

The central hypothesis of this dissertation is that pubertal transition is associated with internalizing symptoms in adolescent males, particularly autistic males who already have emotion regulation challenges. This dissertation addresses three gaps in scientific knowledge. First, I identified risk factors preponderant in typically developing males due to the dearth of studies that examine gender-specific characteristics during puberty. A stark gender difference between males and females in mental health outcomes during puberty has been found (Hayward & Sanborn, 2002; Deardorff et al., 2019), but male-specific pathways that increase risk for internalizing problems are largely understudied. This dissertation addressed this gap by using novel assessment and statistical approaches designed specifically to test links between male puberty and psychopathology symptoms. Second, I examined a theorized yet largely unexamined two-hit model of autism (Picci & Scherf, 2015) that suggests puberty exacerbates autistic stereotypy as well as lead to internalizing comorbidity. Third, I examined differences in autistic-like behaviors and internalizing symptoms between typically developing and autistic adolescent males. Investigating these three knowledge gaps advances understanding of sex as a biological variable by focusing on gender-specific developmental pathways and understudied aspects of male development.

This dissertation utilized three separate datasets to address the three specific aims. Each dataset had strengths and limitations, and the limitations were mainly related to some of the research gaps mentioned above, such as an overlooked subset of vulnerable adolescent males at risk for internalizing disorders. The Wisconsin Twin Project (WTP) dataset included three waves of data on youth, which allowed for longitudinal examination of Aim 1. Aim 1 addressed two research questions: Do males at risk for psychopathology symptoms at an earlier wave continue to show similar symptoms or symptom exacerbation during adolescence, and are these increasing symptoms related to puberty and pubertal hormones? Aim 2 addressed a separate question using the National Database on Autism Research (NDAR) dataset with a sample of autistic children and adolescents and a typically developing comparison group. The NDAR dataset allowed for cross-sectional analyses between autistic and typically developing children and adolescents to examine psychopathology symptoms and autistic behavioral differences between groups and whether these differences are associated with puberty. Lastly, Aim 3 utilized a unique dataset from the Social-Conscious Emotion Study (SCE) of adolescent males in which the study design targeted a comparison in neurobiological differences between typically developing and autistic adolescent males. Similar to the NDAR dataset, the SCE dataset had two comparison groups of adolescent males; however, the SCE dataset included the pubertal hormone testosterone, but the NDAR dataset did not. Though each dataset had its own limitations, the strength of each dataset was by the utility in addressing understudied sets of research questions and examination of gender-differentiated psychopathology during pubertal transition. Therefore, I approached these research questions and aims using the WTP, NDAR, and SCE datasets in three separate papers.

References

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders, 4th ed. - PsycNET* (4th ed.). https://doi.org/doi:10.1176/appi.books.9780890423349

American Psychological Association. (2020). *Bias-Free Language. In Publication Manual of the American Psychological Association, Disability* (7th ed.). Washington, DC.

Angold, Adrian, Erkanli, A., Loeber, R., Costello, E. J., Van KammenN, W. B., & Stouthamer-Loeber, M. (1996). Disappearing Depression in a Population Sample of Boys. *Journal of Emotional and Behavioral Disorders*, *4*(2), 95–104. https://doi.org/10.1177/106342669600400204

Auchus, R. J., & Rainey, W. E. (2004). Adrenarche - physiology, biochemistry and human disease. *Clinical Endocrinology*, *60*(3), 288–296. https://doi.org/10.1046/j.1365-2265.2003.01858.x

Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., … Dowling, N. F. (2018). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR. Surveillance Summaries*, *67*(6), 1–23. https://doi.org/10.15585/mmwr.ss6706a1

Biro, F. M., Pajak, A., Wolff, M. S., Pinney, S. M., Windham, G. C., Galvez, M. P., … Teitelbaum, S. L. (2018). Age of Menarche in a Longitudinal US Cohort. *Journal of Pediatric and Adolescent Gynecology*, *31*(4), 339–345. https://doi.org/10.1016/j.jpag.2018.05.002

Becerra, T. A., von Ehrenstein, O. S., Heck, J. E., Olsen, J., Arah, O. A., Jeste, S. S., … Ritz, B. (2014). Autism spectrum disorders and race, ethnicity, and nativity: a population-based study. *Pediatrics*, *134*(1), e63-71. https://doi.org/10.1542/peds.2013-3928

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., & Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(42), 9228–9231. https://doi.org/10.1523/JNEUROSCI.3340-04.2004

Berkovits, L., Eisenhower, A., & Blacher, J. (2017). Emotion Regulation in Young Children with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *47*(1), 68–79. https://doi.org/10.1007/s10803-016-2922-2

Blakemore, S.-J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human Brain Mapping*, *31*(6), 926–933. https://doi.org/10.1002/hbm.21052

Bordini, B., & Rosenfield, R. L. (2011). Normal pubertal development: part II: clinical aspects of puberty. *Pediatrics in Review*, *32*(7), 281–292. https://doi.org/10.1542/pir.32-7-281

Broome, M. R., He, Z., Iftikhar, M., Eyden, J., & Marwaha, S. (2015). Neurobiological and behavioural studies of affective instability in clinical populations: A systematic review. *Neuroscience & Biobehavioral Reviews*, *51*, 243–254. https://doi.org/10.1016/J.NEUBIOREV.2015.01.021

Byrne, M. L., Whittle, S., Vijayakumar, N., Dennison, M., Simmons, J. G., & Allen, N. B. (2017). A systematic review of adrenarche as a sensitive period in neurobiological development and mental health. *Developmental Cognitive Neuroscience*, *25*, 12–28. https://doi.org/10.1016/J.DCN.2016.12.004

Cabrera, S. M., Bright, G. M., Frane, J. W., Blethen, S. L., & Lee, P. A. (2014). Age of thelarche and menarche in contemporary US females: a cross-sectional analysis. *Journal of Pediatric Endocrinology and Metabolism*, *27*(1–2), 47–51. https://doi.org/10.1515/jpem-2013-0286

Carragher, N., Teesson, M., Sunderland, M., Newton, N. C., Krueger, R. F., Conrod, P. J., … Slade, T. (2016). The structure of adolescent psychopathology: A symptom-level analysis. *Psychological Medicine*. https://doi.org/10.1017/S0033291715002470

Center for Behavioral Health Statistics and Quality. (2016). *2015 National Survey on Drug Use and Health: Summary of the Effects of the 2015 NSDUH Questionnaire Redesign: Implications for Data Users*. (June 2016), 44. Retrieved from https://www.samhsa.gov/data/sites/default/files/NSDUH-TrendBreak-2015.pdf

Chiri, G., & Warfield, M. E. (2012). Unmet Need and Problems Accessing Core Health Care Services for Children with Autism Spectrum Disorder. *Maternal and Child Health Journal*, *16*(5), 1081–1091. https://doi.org/10.1007/s10995-011-0833-6

Cibralic, S., Kohlhoff, J., Wallace, N., McMahon, C., & Eapen, V. (2019, December 1). A systematic review of emotion regulation in children with Autism Spectrum Disorder. *Research in Autism Spectrum Disorders*, Vol. 68. https://doi.org/10.1016/j.rasd.2019.101422

Cicchetti, D., & Rogosch, F. A. (1996). Equifinality and multifinality in developmental psychopathology. *Development and Psychopathology*, *8*(04), 597. https://doi.org/10.1017/S0954579400007318

Costello, J. E., Erkanli, A., & Angold, A. (2006). Is there an epidemic of child or adolescent depression? *Journal of Child Psychology and Psychiatry*, *0*(0), 061024022229003-??? https://doi.org/10.1111/j.1469-7610.2006.01682.x

Courchesne, E. (2004). Brain development in autism: Early overgrowth followed by premature arrest of growth. *Mental Retardation and Developmental Disabilities Research Reviews*, *10*(2), 106–111. https://doi.org/10.1002/mrdd.20020

Courchesne, Eric, Carper, R., & Akshoomoff, N. (2003). Evidence of Brain Overgrowth in the First Year of Life in Autism. *JAMA*, *290*(3), 337. https://doi.org/10.1001/jama.290.3.337

Courchesne, E, Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., … Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology*, *57*(2), 245–254. https://doi.org/10.1212/WNL.57.2.245

Dawson, G., Jones, E. J. H., Merkle, K., Venema, K., Lowy, R., Faja, S., … Webb, S. J. (2012). Early Behavioral Intervention Is Associated With Normalized Brain Activity in Young Children With Autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, *51*(11), 1150–1159. https://doi.org/10.1016/J.JAAC.2012.08.018

Dawson, G., Webb, S. J., & McPartland, J. (2005). Understanding the Nature of Face Processing Impairment in Autism: Insights From Behavioral and Electrophysiological Studies. *Developmental Neuropsychology*, *27*(3), 403–424. https://doi.org/10.1207/s15326942dn2703_6

Deardorff, J., Hoyt, L. T., Carter, R., & Shirtcliff, E. A. (2019). Next Steps in Puberty Research: Broadening the Lens Toward Understudied Populations. *Journal of Research on Adolescence*. https://doi.org/10.1111/jora.12402

Dorn, L. D. (2006). Measuring puberty. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *39*(5), 625–626. https://doi.org/10.1016/j.jadohealth.2006.05.014

Dunn, D. S., & Andrews, E. E. (2015). Person-first and identity-first language: Developing psychologists' cultural competence using disability language. *American Psychologist*, *70*(3), 255–264. https://doi.org/10.1037/a0038636

Durkin, M. S., Maenner, M. J., Baio, J., Christensen, D., Daniels, J., Fitzgerald, R., … Yeargin-Allsopp, M. (2017). Autism Spectrum Disorder Among US Children (2002–2010): Socioeconomic, Racial, and Ethnic Disparities. *American Journal of Public Health*, *107*(11), 1818–1826. https://doi.org/10.2105/AJPH.2017.304032

Essex, M. J., Kraemer, H. C., Armstrong, J. M., Boyce, W. T., Goldsmith, H. H., Klein, M. H., … Kupfer, D. J. (2006). Exploring risk factors for the emergence of children's mental health problems. *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.63.11.1246

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Foster, D. L., Jackson, L. M., & Padmanabhan, V. (2006). Programming of GnRH feedback controls timing puberty and adult reproductive activity. *Molecular and Cellular Endocrinology*, *254–255*, 109–119. https://doi.org/10.1016/J.MCE.2006.04.004

Freed, D., & Pevsner, J. (2016). The Contribution of Mosaic Variants to Autism Spectrum Disorder. *PLoS Genetics*. https://doi.org/10.1371/journal.pgen.1006245

Gernsbacher, M. A. (2017). Editorial Perspective: The use of person-first language in scholarly writing may accentuate stigma. *Journal of Child Psychology and Psychiatry*, *58*(7), 859–861. https://doi.org/10.1111/jcpp.12706

Häggström, M. (2014). Reference ranges for estradiol, progesterone, luteinizing hormone and follicle-stimulating hormone during the menstrual cycle. *WikiJournal of Medicine*, *1*(1). https://doi.org/10.15347/wjm/2014.001

Hayward, C., & Sanborn, K. (2002). Puberty and the emergence of gender differences in psychopathology. *Journal of Adolescent Health*, *30*(4), 49–58. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11943575

Herbison, A. E. (2008). Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: The case for the rostral periventricular area of the third ventricle (RP3V). *Brain Research Reviews*, *57*(2), 277–287. https://doi.org/10.1016/j.brainresrev.2007.05.006

Herbison, A. E. (2016). Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. *Nature Reviews Endocrinology*. https://doi.org/10.1038/nrendo.2016.70

Herman-Giddens, M. E., Wang, L., & Koch, G. (2001). Secondary Sexual Characteristics in Boys. *Archives of Pediatrics & Adolescent Medicine*, *155*(9), 1022. https://doi.org/10.1001/archpedi.155.9.1022

Kokkevi, A., Arapaki, R. A., & Richardson, C. (2011). Adolescents' self-reported suicide attempts, self-harm thoughts and their correlates across 17 European countries. *Ournal of Child Psychology and Psychiatry*, *53*(4), 381–389. https://doi.org/https://doi.org/10.1111/j.1469-7610.2011.02457.x

Kraemer, H. C., Measelle, J. R., Ablow, J. C., Essex, M. J., Boyce, W. T., & Kupfer, D. J. (2003). A new approach to integrating data from multiple informants in psychiatric assessment and research: Mixing and matching contexts and perspectives. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.160.9.1566

Kushki, A., Anagnostou, E., Hammill, C., Duez, P., Brian, J., Iaboni, A., … Lerch, J. P. (2019). Examining overlap and homogeneity in ASD, ADHD, and OCD: a data-driven, diagnosis-agnostic approach. *Translational Psychiatry*, *9*(1), 318. https://doi.org/10.1038/s41398-019-0631-2

Lahey, B. B., Rathouz, P. J., Van Hulle, C., Urbano, R. C., Krueger, R. F., Applegate, B., … Waldman, I. D. (2008). Testing Structural Models of DSM-IV Symptoms of Common Forms of Child and Adolescent Psychopathology. *Journal of Abnormal Child Psychology*, *36*(2), 187–206. https://doi.org/10.1007/s10802-007-9169-5

Louis, G. M., Gray, L. E., Marcus, M., Ojeda, S. R., Pescovitz, O. H., Witchel, S. F., … Euling, S. Y. (2008). Environmental factors and puberty timing: expert panel research needs. *Pediatrics*, *121 Suppl 3*(Supplement 3), S192-207. https://doi.org/10.1542/peds.1813E

Mandell, D. S., Novak, M. M., & Zubritsky, C. D. (2005). Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics*, *116*(6), 1480–1486. https://doi.org/10.1542/peds.2005-0185

Marceau, K., Ram, N., Houts, R. M., Grimm, K. J., & Susman, E. J. (2011). Individual differences in boys' and girls' timing and tempo of puberty: modeling development with nonlinear growth models. *Developmental Psychology*, *47*(5), 1389–1409. https://doi.org/10.1037/a0023838

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*, *44*(235), 291–303. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5785179

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood*, *45*(239), 13–23. https://doi.org/10.1136/ADC.45.239.13

Martel, M. M., Klump, K., Nigg, J. T., Breedlove, S. M., & Sisk, C. L. (2009). Potential hormonal mechanisms of attention-deficit/hyperactivity disorder and major depressive disorder: a new perspective. *Hormones and Behavior*, *55*(4), 465–479. https://doi.org/10.1016/j.yhbeh.2009.02.004

Matchock, R. L., Dorn, L. D., & Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty. *Chronobiology International*. https://doi.org/10.1080/07420520701649471

Marwaha, S., He, Z., Broome, M., Singh, S. P., Scott, J., Eyden, J., & Wolke, D. (2014). How is affective instability defined and measured? A systematic review. *Psychological Medicine*, *44*(9), 1793–1808. https://doi.org/10.1017/S0033291713002407

Mazefsky, C. A., Herrington, J., Siegel, M., Scarpa, A., Maddox, B. B., Scahill, L., & White, S. W. (2013). The role of emotion regulation in autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(7), 679–688. https://doi.org/10.1016/j.jaac.2013.05.006

McLaughlin, K. A., Aldao, A., Wisco, B. E., & Hilt, L. M. (2014). Rumination as a transdiagnostic factor underlying transitions between internalizing symptoms and aggressive behavior in early adolescents. *Journal of Abnormal Psychology*. https://doi.org/10.1037/a0035358

Mendle, J., & Ferrero, J. (2012). Detrimental psychological outcomes associated with pubertal timing in adolescent boys. *Developmental Review*, *32*(1), 49–66. https://doi.org/10.1016/J.DR.2011.11.001

Negriff, S., Fung, M. T., & Trickett, P. K. (2008). Self-Rated Pubertal Development, Depressive Symptoms and Delinquency: Measurement Issues and Moderation by Gender and Maltreatment. *Journal of Youth and Adolescence*, *37*(6), 736–746. https://doi.org/10.1007/s10964-008-9274-y

Neuhaus, E., Webb, S. J., & Bernier, R. A. (2019). Linking social motivation with social skill: The role of emotion dysregulation in autism spectrum disorder. *Development and Psychopathology*, 1–13. https://doi.org/10.1017/S0954579419000361

Noordhof, A., Krueger, R. F., Ormel, J., Oldehinkel, A. J., & Hartman, C. A. (2015). Integrating Autism-Related Symptoms into the Dimensional Internalizing and Externalizing Model of Psychopathology. The TRAILS Study. *Journal of Abnormal Child Psychology*. https://doi.org/10.1007/s10802-014-9923-4

Orentreich, N., Brind, J. L., Rizer, R. L., & Vogelman, J. H. (1984). Age Changes and Sex Differences in Serum Dehydroepiandrosterone Sulfate Concentrations throughout Adulthood. *The Journal of Clinical Endocrinology & Metabolism*, *59*(3), 551–555. https://doi.org/10.1210/jcem-59-3-551

Perou, R., Bitsko, R. H., Blumberg, S. J., Pastor, P., Ghandour, R. M., G.C., J., … Huang, L. N. (2013). Mental Health Surveillance Among Children — United States, 2005–2011. *Morbidity and Mortality Weekly Report*, *62*(2), 2005–2011. Retrieved from https://www.cdc.gov/Mmwr/preview/mmwrhtml/su6202a1.htm

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissue mediating mating behavior in the female guinea pig. *Endocrinology*, *65*(3), 369–382. https://doi.org/10.1210/endo-65-3-369

Picci, G., & Scherf, K. S. (2015). A Two-Hit Model of Autism: Adolescence as the Second Hit. *Clinical Psychological Science*, *3*(3), 349–371. https://doi.org/10.1177/2167702614540646

Rodriguez-Seijas, C., Gadow, K. D., Rosen, T. E., Kim, H., Lerner, M. D., & Eaton, N. R. (2019). A transdiagnostic model of psychiatric symptom co-occurrence and autism spectrum disorder. *Autism Research*, aur.2228. https://doi.org/10.1002/aur.2228

Rogol, A. D. (1992). Growth and growth hormone secretion at puberty: the role of gonadal steroid hormones. *Acta Paediatrica (Oslo, Norway : 1992). Supplement*, *383*, 15–20; discussion 21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1458010

Romeo, R. D. (2003). Puberty: A Period of Both Organizational and Activational Effects of Steroid Hormones On Neurobehavioural Development. *Journal of Neuroendocrinology*, *15*(12), 1185–1192. https://doi.org/10.1111/j.1365-2826.2003.01106.x

Rubinow, D. R., & Schmidt, P. J. (1996). Androgens, brain, and behavior. *American Journal of Psychiatry*, *153*(8), 974–984. https://doi.org/10.1176/ajp.153.8.974

Ruch, D. A., Sheftall, A. H., Schlagbaum, P., Rausch, J., Campo, J. V., & Bridge, J. A. (2019). Trends in Suicide Among Youth Aged 10 to 19 Years in the United States, 1975 to 2016. *JAMA Network Open*, *2*(5), e193886. https://doi.org/10.1001/jamanetworkopen.2019.3886

Samson, A. C., Phillips, J. M., Parker, K. J., Shah, S., Gross, J. J., & Hardan, A. Y. (2014). Emotion Dysregulation and the Core Features of Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *44*(7), 1766–1772. https://doi.org/10.1007/s10803-013-2022-5

Schalock, R. L., Luckasson, R. A., & Shogren, K. A. (2009). The Renaming of Mental Retardation: Understanding the Change to the Term Intellectual Disability. *Http://Dx.Doi.Org/10.1352/1934-9556(2007)45[116:TROMRU]2.0.CO;2*. https://doi.org/10.1352/1934-9556(2007)45[116:TROMRU]2.0.CO;2

Schulz, K. M., Molenda-Figueira, H. A., & Sisk, C. L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior*, *55*(5), 597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology*. https://doi.org/10.1002/dev.20336

Simerly, R. B., Swanson, L. W., Chang, C., & Muramatsu, M. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. *The Journal of Comparative Neurology*, *294*(1), 76–95. https://doi.org/10.1002/cne.902940107

Sisk, C. L., & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature Neuroscience*, *7*(10), 1040–1047. https://doi.org/10.1038/nn1326

Steinberg, L. (2004). Risk Taking in Adolescence: What Changes, and Why? *Annals of the New York Academy of Sciences*, *1021*(1), 51–58. https://doi.org/10.1196/annals.1308.005

Wallen, K. (2009). The Organizational Hypothesis: Reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). *Hormones and Behavior*, *55*(5), 561–565. https://doi.org/10.1016/J.YHBEH.2009.03.009

Walton, K. E., Ormel, J., & Krueger, R. F. (2011). The Dimensional Nature of Externalizing Behaviors in Adolescence: Evidence from a Direct Comparison of Categorical, Dimensional, and Hybrid Models. *Journal of Abnormal Child Psychology*, *39*(4), 553–561. https://doi.org/10.1007/s10802-010-9478-y

Weiss, J. A., Isaacs, B., Diepstra, H., Wilton, A. S., Brown, H. K., McGarry, C., & Lunsky, Y. (2018). Health Concerns and Health Service Utilization in a Population Cohort of Young Adults with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *48*(1), 36–44. https://doi.org/10.1007/s10803-017-3292-0

Winkler, I. T., & Roaf, V. (2015). Taking the Bloody Linen Out of the Closet: Menstrual Hygiene As a Priority for Achieving Gender Equality. *Cardozo Journal of Law & Gender*.

Zahn-Waxler, C., Crick, N. R., Shirtcliff, E. A., & Woods, K. E. (2006). *Developmental Psychopathology*. John Wiley & Sons, Inc.

Zahn-Waxler, C., Crick, N. R., Shirtcliff, E. A., & Woods, K. E. (2015). The Origins and Development of Psychopathology in Females and Males. In *Developmental Psychopathology* (pp. 76–138). https://doi.org/10.1002/9780470939383.ch4

Zahn-Waxler, Carolyn, Shirtcliff, E. A., & Marceau, K. (2008). Disorders of Childhood and Adolescence: Gender and Psychopathology. *Annual Review of Clinical Psychology*, *4*(1), 275–303. https://doi.org/10.1146/annurev.clinpsy.3.022806.091358

Zahn-Waxler, C, Klimes-Dougan, B., & Slattery, M. J. (2000). Internalizing problems of childhood and adolescence: prospects, pitfalls, and progress in understanding the development of anxiety and depression. *Development and Psychopathology*, *12*(3), 443–466. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11014747

Zawatski, W., & Lee, M. M. (2013). Male pubertal development: are endocrine-disrupting compounds shifting the norms? *Journal of Endocrinology*, *218*(2), R1–R12. https://doi.org/https://doi.org/10.1530/JOE-12-0449

CHAPTER 3. THE ROLE OF PUBERTY AND HORMONES IN PSYCHOPATHOLOGY SYMPTOMS SEVERITY AND DIRECTIONALITY IN MALE ADOLESCENTS ACROSS DEVELOPMENT

Modified from a manuscript to be submitted to the *Journal of Adolescent Health*

Jenny M. Phan[1], Carol Van Hulle[2], Elizabeth A. Shirtcliff[1], & H. Hill Goldsmith[2]

3.1 Author Contributions

In order, the authors of the first manuscript are Jenny M. Phan, Elizabeth A. Shirtcliff, Carol Van Hulle, and H. Hill Goldsmith. Jenny Phan completed the writing and statistical analysis of the present study. Coauthors provided statistics consultation, reviewed, and edited drafts of the manuscript. Hill Goldsmith is the principal investigator of the Wisconsin Twin Project.

3.2 Abstract

Longitudinal studies on psychopathology symptoms stability and change in male adolescents are necessary for understanding developmental processes linked to symptoms severity. In this study, male children and adolescents were tracked from ages 5 to 18 years. Data on Tanner staging, the Pubertal Development Scale, pubertal hormones (dehydroepiandrosterone [DHEA], testosterone), and a range of psychopathology measures were collected. Growth curve models combined with an accelerated longitudinal design were utilized to examine the role of pubertal status and pubertal hormones on male adolescents' symptoms severity stability or change and the directionality between internalizing (depression, anxiety) and externalizing (conduct problems, oppositional defiant behaviors, overt aggression) symptoms. Symptoms severity was driven by pubertal timing within individuals, and symptoms directionality was driven by developmental age. Early developing male adolescents had increasing symptoms severity, and as male youth aged while controlling for puberty, they showed a preponderance of

externalizing symptoms. High DHEA level was associated with lowered symptoms severity, while high testosterone level was associated with elevated symptoms severity and showed a preponderance of externalizing symptoms. Findings from this study suggest that some male adolescents are more vulnerable to normative changes during puberty and as they matured. Future research is needed on investigating risk factors that put some male adolescents into risk categories for increasing psychopathology symptoms over time and a preponderance of psychopathologies.

---

[1] Iowa State University, Department of Human Development and Family Studies, Ames, Iowa

[2] University of Wisconsin-Madison, Department of Psychology, Waisman Center, Madison, Wisconsin

3.3 Introduction

Internalizing disorders, such as depression, anxiety, and mood problems, affect adolescents at an alarming rate (CDC, 2020). While internalizing disorders affect both males and females, males with internalizing symptoms may go undetected because they can present symptoms differently than females (Khesht-Masjedi, Shokrgozar, Abdollahi, Golshahi, & Sharif-Ghaziani, 2017). Males typically have higher rates of externalizing symptoms (Afzali, Sunderland, Carragher, & Conrod, 2018), such as attention deficit hyperactivity disorder (Rucklidge, 2010), conduct disorder (McCabe, Rodgers, Yeh, & Hough, 2004), oppositional defiant disorder (López-Villalobos et al., 2014), and antisocial personality disorder (Sher et al., 2015), than internalizing symptoms. Regardless of male preponderance of externalizing symptoms, a subset of male adolescents experiences internalizing symptoms, and the process at which internalizing symptoms development occur is still unknown. Heterotypic comorbidity of

symptoms in adolescent males (presentation in both internalizing and externalizing domains) has been found in several studies (Burke, Loeber, Lahey, & Rathouz, 2005; Lahey et al., 2008). Detection of increasing internalizing symptoms in adolescent males will involve carefully examining cross-psychopathology domains, especially crossing the male-preponderant psychopathology categories, to uncover when internalizing symptoms begin and how it relates to other psychopathology domains.

Developmental onset of psychopathology symptoms can begin in early childhood and/or during adolescence (Zahn-Waxler et al., 2008; Zahn-Waxler et al., 2015). Elevated psychopathology can have developmental onset, a waning period, second onset, and another waning period throughout an individual's lifespan, more specifically with internalizing symptoms (McLaughlin & King, 2014). Context contributes largely to certain psychological disorders displaying peak in symptoms, for instance, in ongoing chronic conditions (e.g., pain, stress; Blackburn-Munro & Blackburn-Munro, 2001; Fishbain, Cutler, Rosomoff, & Rosomoff, 1997), during certain seasons (e.g., winter; Morken, Lilleeng, & Linaker, 2002), and in life events (death in family, residential relocation, divorce; Kessler, 2013). In order to better determine switch-points of onset and waning (or fluctuating trajectory) of a psychological disorder, several factors and/or points must be considered. We begin with the utilization of longitudinal models as a tool for addressing symptoms severity change or stability across development.

Longitudinal models, such as growth curve models, allow tracking onset and stability of symptoms within- and between-individuals. Growth curve models (GCMs) are beneficial because it allows to track variability in childhood onset and later onset of psychopathology symptoms to see distinctions within a person across time. In this study, we utilized GCM to track

psychopathology symptoms change or stability within males as they develop from childhood to adolescence. Using GCMs and selecting only one gender (e.g., males) allowed for the potential to target an overlooked gender when studying psychopathology symptoms that may be missed in gender differences studies. Lastly, another important consideration is comorbidity of externalizing and internalizing symptoms and adopting a transdiagnostic approach to modeling psychopathology. Some may suggest a symptom-level analysis (Carragher et al., 2016) or a diagnosis-agnostic approach that is data-driven (Kushki et al., 2019). Another method is a severity-directionality symptoms analysis in which severity of combined internalizing and externalizing symptoms can be tested as well as the directionality of symptoms—a preponderance of a discrete psychopathology domain (Essex et al., 2006; Kraemer et al., 2003; Shirtcliff & Essex, 2008). Given that a subset of males has both externalizing and internalizing symptoms, this strategy opens up the possibility to examine change or stability of symptoms severity and directionality over time, and an example of such observation is childhood to adolescent development as youth undergo puberty.

All adolescents undergo puberty, and puberty is a normative developmental process, but for some adolescents, physical changes can come with psychosocial challenges. Adolescents' experiences with pubertal maturation can vary and those experiences may be related to earlier exposure to psychopathology symptoms, their gender, underlying neuroendocrine function, and/or pubertal timing (pubertal status relative to age-matched youth). Pubertal timing has been found as a marker of internalizing symptoms increase in some females (Mendle, Turkheimer, & Emery, 2007; Marceau et al., 2011), and a smaller subset of males with increasing internalizing symptoms during puberty receive little attention (Graber, 2013; Mendle & Ferrero, 2012). This may also be due to limited research and understanding on male pubertal maturation (Deardorff et

al., 2019). Physical maturation (e.g., testes growth size) is less visible in males during the early stages of maturation (compared to females) until Tanner stage 4 (Herman-Giddens et al., 2001; Marshall & Tanner, 1970). Therefore, much of male pubertal development may go unnoticed and perhaps even a disassociation of arising psychopathology symptoms severity that could be attributed to puberty.

Adrenarche, a developmental period of neuroendocrine activation driven by high levels of the hormone dehydroepiandrosterone (DHEA) and its sulfate form DHEA-S released from the adrenal cortex (Havelock, Auchus, & Rainey, 2004; Byrne et al., 2017), may be an important developmental event for males who show increasing psychopathology symptoms. DHEA is associated with adrenarcheal-related changes during puberty (e.g., body odor, oily skin). Elevated DHEA levels are expected during adrenarche (Guran et al., 2015) and initiates the beginning of gonadarche. Activation of the hypothalamic-pituitary-gonadal (HPG) axis initiates gonadarche, and in males, this means enlargement of the testes and more pubic hair growth (among other secondary sexual characteristics). Testosterone is associated with secondary sexual characteristic changes that are typically more visible in males. DHEA's and testosterone's role in pubertal maturation may also play a part in psychopathology symptoms development. This topic is relatively understudied, particularly DHEA and psychopathology symptoms in adolescents. One study found an association between high DHEA levels during adrenarche and elevated externalizing symptoms in females (Whittle et al., 2014), and another study found an association between high DHEA levels and depressive symptoms in both males and females (Goodyer, Herbert, Tamplin, & Altham, 2000). For testosterone, one study found lower levels of testosterone was linked to elevated anxiety and depressive symptoms in males (Granger et al.,

2003) and that high levels of testosterone was associated with elevated externalizing symptoms in adolescent males (Maras et al., 2003)

With a focus on male child and adolescent development over time, investigation of psychopathology symptoms severity and directionality could be the next step in gaining knowledge in risks associated with psychopathology development in males. Along with this investigation, an analysis of pubertal timing's and pubertal hormones' effect on psychopathology symptoms in males can also be another view into the neurobiological changes that impact adolescent psychophysiology. In the present study, due to adjacent ages in each cohort across three waves of data collection, we used a cohort sequential design to examine population deviation trends measured by age and puberty as well as within-individual developmental trajectory captured by measured time.

3.3.1 Study Aims and Hypotheses

The present study aimed to investigate the role of pubertal development and hormones in psychopathology symptoms development in males using a transdiagnostic approach. A growth curve model accelerated longitudinal design was used to test symptoms severity and directionality between internalizing and externalizing symptoms domain across development, which allowed to examine stability or change in symptoms severity and directionality and at which point of development symptoms were elevated or changed directional domain. To our knowledge, this is the first study to use accelerated longitudinal design to investigate psychopathology symptoms severity and directionality in males across child and adolescent development. We then investigated if pubertal timing and pubertal hormones influenced symptoms severity and directionality over time. We hypothesized male adolescents show change in symptoms severity over time as they mature in age and undergo puberty. We hypothesized

that an age effect and a quadratic effect of age show symptoms severity change as was previously found by McLaughlin & King (2014). We hypothesized that male youth show more externalizing than internalizing symptoms earlier in development but then switch domain to more internalizing than externalizing symptoms later in development (Zahn-Waxler et al., 2008). We expect symptom severity to peak for early developers because drastic physical maturation becomes apparent and is likely more noticeable relative to their peers. We hypothesized that some male adolescents show more internalizing symptoms based on their pubertal timing. We hypothesized that male youth who are early developers relative to other youth their age shows more externalizing symptoms than internalizing symptoms, and vice versa, that later developers show more internalizing than externalizing symptoms. We hypothesized that DHEA and testosterone levels have an effect on increasing male adolescents' symptoms severity; specifically, male youth with high testosterone levels show more externalizing symptoms, and male youth with high DHEA levels show more internalizing symptoms.

3.4 Methods

3.4.1 Participants

Participants were recruited from a Wisconsin Twin Study from birth and were followed up to when they were approximately 16 to 18 years old (see Schmidt et al., 2013 for recruitment details). This was a longitudinal study of twins between 1989 and 2004. Five time points were collected in the larger study within the same sample of twins. The present study focused on wave 3 (M age = 7.44 years [SD = 0.96], N = 1711), wave 4 (M age = 13.16 years [SD = 1.26], N = 348), and wave 5 (M age = 14 years [SD = 1.34], N = 387) that tracked psychopathology symptoms from childhood to adolescence. Mothers and fathers were invited to participate also as multiple informants on corresponding questionnaires on youths' (and family's) demographics,

puberty, and psychopathologies. When assessment took place at wave 3, family income was on average $60,000-$70,000, and income amount remained stable across all three waves. Mothers and fathers on average had formal schooling at either trade, technical, or some college (mothers: M = 14.81 years, SD = 2.34; fathers: M = 14.45 years, SD = 2.38). The focus of the present study was on male adolescents and growth and development of psychopathology symptoms over time; therefore, females were excluded from the present study. Youth were predominantly non-Hispanic white (see Schmidt et al., 2013 for demographic details). Adolescents provided saliva samples that were later assayed for cortisol, testosterone, and DHEA. Only pubertal hormones (testosterone, DHEA) were included in the present study. All study protocols were approved by the University of Wisconsin—Madison Institutional Review Board. Parents signed informed consent forms as well as assent from youth were obtained at each assessment. Families were compensated for their participation.

3.4.2 Measures of Internalizing and Externalizing Symptoms

Each measure was collected at wave 3, 4, and 5, with the exception of puberty and hormone measures (collected only during wave 4 and 5). We will refer to waves as Time 1, 2, and 3 throughout the remainder of this paper and to set the first timepoint by the age at which data collection for wave 3 began as well as to simplify time interpretations in the results. Three surveys were included in the present study for internalizing and externalizing symptoms. The Child Depression Inventory (mother and father reports; CDI, Timbremont, Braet, & Dreessen, 2004; Kovacs, 2010) was used as a measure of depressive symptoms and has been found a reliable measure for depressive symptoms in children ages 7-16 years with good test-retest reliability (Timbremont et al., 2004). Construct (Doerfler, Felner, Rowlison, Raley, & Evans, 1988) and discriminant (Hodges, 1990) validity has also been established. The second survey is

the Multidimensional Anxiety Scale for Children (adolescent reports; MASC, March, 2004), a measure of anxiety symptoms in children ages 8 to 19 years and captures emotional, physical, cognitive, and behavioral symptoms of anxiety, which also has good reliability and validity (March, Parker, Sullivan, Stallings, & Conners, 1997) and test-retest reliability (March, Sullivan, & Parker, 1999). The third survey is the Health and Behavior Questionnaire, Symptoms Scale (mother and child/adolescent reports; HBQ) focusing on relevant internalizing and externalizing HBQ subscales in the present study, which included measures of depressive, anxiety, conduct disorder, oppositional defiant disorder, and overt aggressive symptoms. The HBQ has a strong test-retest reliability and discriminant validity (Ablow et al., 1999) on the symptoms scales and is a valid measure of youth mental health symptoms (Lemery-Chalfant et al., 2007). See Table 3.9.1 for psychopathology measures' descriptive statistics.

A multimethod approach with multi-measures and multi-informants was used to combine psychopathology symptoms measures for each domain. This approach was used to account for informant reliability of reported symptoms. First, the CDI was combined with the depression subscale from the HBQ as an average score of depressive symptoms. The MASC measure was combined with the anxiety subscales of the HBQ in an average score of anxiety symptoms for each individual. The anxiety subscales were combined as an average score. Scores for conduct disorder, oppositional defiant disorder, and overt aggression HBQ subscales were converted into separate average scores and represented externalizing symptoms. Each psychopathology average scores (depression, anxiety, conduct disorder, oppositional defiant disorder, overt aggression) were standardized within informant (father, mother, youth).

Severity and directionality scores have been used in previous studies (Kraemer et al., 2003; Essex et al., 2006; Shirtcliff & Essex, 2008). Severity and directionality scores were

derived from principal component analysis (PCA). PCAs of psychopathology scores were run separately for Time 1, Time 2, and Time 3. We fixed the number of components to 2 for a severity component and a directionality component. For Time 1, Component 1 (severity) had an Eigenvalue of 2.999 and explained 59.98% of the total variance; Component 2 (directionality) had an Eigenvalue of 0.932 and explained 18.64% of the total variance. For Time 2, Component 1 (severity) had an Eigenvalue of 2.87 and explained 57.41% of the total variance; Component 2 (directionality) had an Eigenvalue of 0.961 and explained 19.22% of the total variance. For Time 3, Component 1 (severity) had an Eigenvalue of 2.467 and explained 49.34% of the total variance; Component 2 (directionality) had an Eigenvalue of 1.464 and explained 29.29% of the total variance. Component loadings are displayed in Table 3.9.2.

Severity and directionality scores were extracted from the PCAs using the regression factoring method. For severity factor scores, higher values represented higher severity in symptoms in both domains (internalizing and externalizing). The standardized severity scores were then converted to an above zero scale by adding a constant (+5) for primary analysis and ease in results interpretation; a severity score of 5 would thus indicate the average level of psychopathology symptoms for that wave. For the directionality factor scores, negative scores represented a preponderance of externalizing symptoms, and positive scores represented a preponderance of internalizing symptoms. Standardized directionality scores were not converted into a difference scale metric in order to interpret results and to determine deviations above or below zero to mean more externalizing or more internalizing.

3.4.3 Measures of Puberty

Three multi-method assessment tools of puberty that included Tanner staging, parent- and self-reported pubertal development, and puberty hormones are used in this study to assess the

process of puberty. Puberty assessments were conducted at Time 2 and Time 3. Due to low number of assessments at Time 2 and funding-related decisions, some youth puberty assessments were not attainable at Time 2. With just slightly over 1 year apart between Time 2 and Time 3, puberty measures were collected for youth at Time 3 who did not provide puberty measures at Time 2.

To capture Tanner stages (Tanner, 1962; Morris & Udry, 1980), the Picture-Based Interview about Puberty (PBIP) was assessed when youth were approximately at 13 years old at Time 2 and Time 3, respectively. This measure showed good reliability (including cross cultures; Norris & Richter, 2005; Rabbani et al., 2013) and a valid measure of pubertal development (Coleman & Coleman, 2002). The Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) was also administered. Using a coding syntax developed by Shirtcliff and colleagues (2009), we calculated a unique pubertal stage score that maps onto the Tanner stage scale metric. On average, male adolescents were mean pubertal stage 2.78 (SD = 0.96, range 1.00-4.88). Tanner stages and the pubertal development stage were standardized within measure and then averaged to form a puberty score. A pubertal timing variable was calculated by regressing youths' age at the time of puberty measures collected on the puberty score and then saved the residuals (Mendle, Beltz, Carter, & Dorn, 2019). Unstandardized residual scores were then used for primary analyses.

Saliva samples were collected at Time 2 (30 minutes after waking across two different days) and later analyzed for the hormones DHEA and testosterone using enzyme-immunoassays (Salimetrics, LLC); hormone assays information is provided in a previous paper (Van Hulle, Moore, Shirtcliff, Lemery-Chalfant, & Goldsmith, 2015). These hormones are of adrenal or gonadal origin and trigger physical growth and maturation of primary and secondary sexual

development (Black, Lerner, Shirtcliff, & Klein, 2018; Sizonenko & Paunier, 1975; Van Hulle, Moore, Shirtcliff, Lemery-Chalfant, & Goldsmith, 2015; Shirtcliff et al., 2009). Youths' mean DHEA morning levels were 157.29 pg/mL (SD = 137.92) on Day 1 and 153.29 pg/mL (SD = 142.54) on Day 2. Morning testosterone mean levels were 71.76 pg/mL (SD = 47.34) on Day 1 and 69.75 pg/mL (SD = 45.43) on Day 2. Outliers were winsorized. Hormone values were natural log transformed due to skewness. Then, the log transformed hormone values were regressed on time since waking to account for any variance due to high waking hormone levels. Finally, hormones between the two days were averaged as a single variable for testosterone and for DHEA.

3.4.4 Analytic Strategy

Hypotheses were tested using growth curve model accelerated longitudinal design (also called cohort sequential model) in Hierarchical Linear Modeling (HLM: Raudenbush, S.W., Bryk, A.S., & Congdon, 2019), also called multilevel modeling. HLM was selected because of its capability to include cases with missing a repeated measure for one wave and still include such a case. Additionally, HLM allows for measurements of adjacent age across cohorts (Moerbeek, 2011), and suited with this longitudinal data, there are youth who overlapped in ages across waves by design. Moerbeek (2011) provided a detailed description of the utility of accelerated longitudinal design, which is fitting for the present study. Moreover, growth curve models are a common statistical tool for examining longitudinal change in an outcome over time and across development (Hoffman, 2013). We combined growth curve modeling and cohort sequential modeling in order to test symptoms severity and directionality change or stability over time and across development in male youths.

In a two-level HLM, Level 1 of the equation provides the intercept (π_{0i}) and slope change/stability of symptoms severity and directionality within an individual when time is added to the model. Time is centered at Time 1 and in sequential years (coded as 0, 5.71, 6.76 years). The outcome variable is a repeated measure (e.g., symptom severity at three timepoints). Below is the Level 1 Model for examining symptoms severity.

$$SEVERITY_{ti} = \pi_{0i} + \pi_{1i}*(YEARS_{ti}) + e_{ti}$$

Level 2 of the equation that includes between-individual predictors (e.g., pubertal timing, hormones) provide parameters of change/stability in symptoms and (co)variance component estimates of any unexplained variance (r_0) ascribed to the Level 2 predictors (Willett, Singer, & Martin, 1998).

$$\pi_{0i} = \beta_{00} + \beta_{01}*(L2PREDICTOR_i) + r_{0i}$$

$$\pi_{1i} = \beta_{10} + \beta_{11}*(L2PREDICTOR_i) + r_{0i}$$

Using an accelerated longitudinal design, age was entered as a Level 2 predictor. In order to capture symptom change or stability over time across development, age was calculated as a change score centered at age in which youth were at Time 1. The age change score was centered at age 6 years due to a large number of youths who were approximately 6 years old at Time 1 with only a few youths who were 5 years old (N = 5) at Time 1. With age as a change score centered at Time 1 and entered as a Level 2 predictor, a baseline of symptoms (or intercept, π_{0i}) is determined at Time 1 (π_{1i}) and at age 6 years old (β_{10}). Age entered at π_{0i} provides the parameter estimate for unit change or stability in symptoms severity within an individual as well as describe the intra-individual variance (e_{ti}) ascribed to an individual's symptoms severity change/stability. Age entered at π_{1i} (cross-level interaction with YEARS) describes the slope change (or no change) in symptoms severity both over time and across development (or age);

this parameter estimate also represents between-individual variance (r_{0i}) accounting for age differences.

We calculated intra-class correlations of the HLM null model—a model with no predictors and just the outcome variable. Of the total variance, within-individual variance was 57% and between-individual variance was 43% in explaining severity symptoms. For explaining directionality of symptoms, 65% of the total variance was within-individual variances, and 35% of the total variance was between-individual variances. Intra-class correlations are useful in understanding the extent to which symptoms severity and directionality vary between adolescent males, and vice versa, whether variance is higher within individuals than between adolescent males. In this sample, symptoms severity and directionality varied more within-individual than between male adolescents. Missing data were treated as pairwise deletions within HLM.

3.5 Results

3.5.1 Puberty and Hormones Predict Symptoms Severity over Time in Male Adolescents

Statistical results are presented in Table 3.9.3. A scatterplot of symptom severity is displayed in Figure 3.10.1. Dots represent deviations from the mean in symptoms severity. We, first, tested a Level 1 model on whether YEARS predicted symptoms severity. YEARS can be interpreted as a within-individual predictor of the trajectory of symptom change over time. The Level 1 predictor YEARS moderately predicted symptoms severity. Symptoms severity was found to decrease over time within-individuals. Models were loaded with Level 2 predictors at the intercept and slopes on symptoms severity, and Level 2 predictors (pubertal timing, hormones) were removed from the model that did not show significant effects. Parsimonious results are reported below.

With age entered as a Level 2 predictor, the trend effect of YEARS became a significant effect in which symptoms severity still decreased over time within youth as they got older, and in particular, youth who were older at the start of the study had greater symptom severity. Symptoms severity did not further significantly change within individuals over time by age and did not significantly show slope change over time by age; put another way, age did not impact the trajectory of symptoms severity over time (i.e., cross-level interaction between YEARS and age). Since age appeared to influence symptoms severity at the within-individual level, we tested a quadratic effect of age on symptoms severity by entering an age x age term on Level 2. Both age and quadratic age were significant predictors of within-individual symptoms severity change. Age showed a positive unit change in symptoms, while quadratic age showed a negative unit change in symptoms indicating that youth who were older at the start of the trajectory had greater symptoms severity, but this effect became less pronounced amongst the oldest youth within Time 1.

Next, we tested whether pubertal timing, as a covariate with age and quadratic age, was a predictor of symptoms severity. Pubertal timing significantly predicted symptoms severity within individuals showing a positive unit change in severity level, which showed that youth who developed earlier than others had greater symptoms severity. Age and quadratic age effects became non-significant and the parameter estimate (or effect size) of symptoms severity at the intercept increased by 0.162 units, which indicated pubertal timing accounted for some of the explained variance in symptoms severity within individuals. Visual representation of this result is shown in Figure 3.10.2. Youth ages at which they were at Time 1 were selected at random to display symptoms severity trajectory designated by pubertal timing categories: late developer, early developer, and average developer. A late developer was an adolescent whose pubertal

status indicated less developed than age-matched adolescents. An early developer was an adolescent whose pubertal status was more developed than age-matched adolescents. Those who were categorized as average developers were adolescents who were developing the same as age-matched adolescents. Early developers showed most elevated symptoms severity compared to average and late developers. Some youth had a trajectory of symptoms increase from when they were younger to when they matured, and this was apparent for late developers.

Lastly, we tested whether pubertal hormones individually predicted symptoms severity by including DHEA and testosterone separately in the GCM. Individually, DHEA and testosterone did not significantly predict symptoms severity at the within- and between-individual levels (ps > 0.099). Given the dual function of DHEA and testosterone during puberty, we, then, examined whether pubertal hormones simultaneously, as covariates with age and quadratic age, were predictors of symptoms severity. There was no significant effect of quadratic age on symptoms severity, and was, therefore, removed from the GCM. On the intercept, DHEA and testosterone were both significant predictors of within-individual symptoms severity change. DHEA showed a negative unit change in symptoms severity demonstrating youth with high morning DHEA level predicted lower symptoms severity, beyond the effect of age. Testosterone had a positive unit change in symptoms severity, which indicated that youth with high morning testosterone level had higher symptoms severity. When examining the effect of within-individual trajectories (across years), DHEA and testosterone only showed a moderate (trend) effect on symptoms severity. Youth on average had declining symptoms over time (YEARS), but for some youth with higher morning DHEA level, they had a slower decrease in symptoms severity over time and that was explained by those with high morning DHEA level who were younger and had fewer symptoms than other youth at Time 1. A trend effect of testosterone was also found at the

cross-level interaction between YEARS and age and showed moderate faster decrease in symptoms severity over time indicating that some youth with high morning testosterone and were younger than other youth and had higher symptoms at Time 1 showed somewhat more decreasing symptoms over time. A visual display of this finding is shown in Figure 3.10.3. Symptoms severity trajectory of youth with starting age at Time 1 and labeled by hormone level profiles: 1) high DHEA and high testosterone, 2) low DHEA and low testosterone, 3) high DHEA and low testosterone, and 4) low DHEA and high testosterone, are depicted in the figure. Youth who had low morning DHEA and high morning testosterone profile had the highest symptoms severity compared to the other hormone profiles, and these youth showed a moderate decrease in symptoms severity over time. Youth who had high DHEA and low testosterone profile had the lowest symptoms severity than other hormone profiles but showed a moderately steeper increase in symptoms severity as they matured.

3.5.2 Puberty and Hormones Predict Directionality of Symptoms over Time in Male Adolescents

Statistical results are presented in Table 3.9.4. A scatterplot of symptoms directionality is shown in Figure 3.10.4. Dots deviating above zero indicate a preponderance of symptoms in the internalizing domain, and dots deviating below zero indicate preponderance of symptoms in the externalizing domain. We, first, tested a Level 1 model on whether YEARS predicted symptom directionality. The Level 1 predictor YEARS did not significantly predict symptom directionality. Symptom directionality appeared to remain stable over time at the within-individual level, which indicated that youth maintained a relatively consistent ranking in symptom type as they mature across years. As mentioned earlier, the intra-class correlation of explained variance was 65% of the total variance attributed to within individuals, and 35% of the total variance was attributed to between-individual variances. An investigation of some of the

explained variance due to within- and/or between-individuals was conducted by examining age, pubertal timing, and pubertal hormones as predictors of symptom directionality.

We investigated change and stability of symptom directionality across time and by age. In the overall sample, youth generally showed moderately more externalizing symptoms than internalizing symptoms. However, age showed a significant effect on symptom directionality within individuals and had a positive unit change in symptom directionality. This indicated that youth who were older than other youth at Time 1 changed in symptom type. If unit change crossed above zero threshold, then symptom directionality has switched into the internalizing domain.

We examined pubertal timing, as a covariate with age and quadratic age, as predictor of symptoms directionality. No significant predictors were found in this GCM. Since quadratic age did not significantly predict symptom directionality, it was removed from the GCM. The final GCM included age and pubertal timing as predictors of symptom directionality. With pubertal timing entered in the GCM, age was a significant predictor of symptom directionality, indicating that youth who were older at Time 1 were more likely to show a preponderance of internalizing symptoms compared to youth who were younger at the start of the study who were more likely to show a preponderance of externalizing symptoms. Pubertal timing did not significantly predict symptom directionality. When pubertal timing was removed from the GCM, age then became non-significant. Pubertal timing functionally was a suppressor variable of the age effect in predicting symptom directionality. Figure 3.10.5 shows across the time trajectory, no strong preponderance in symptom directionality was found, accounting for pubertal timing. However, across each of the pubertal status categories, youth showed a preponderance of externalizing

symptoms, as was previously mentioned that pubertal timing did not significantly predict symptom directionality.

Lastly, we tested whether pubertal hormones were significant predictors of symptom directionality and as covariates with age. Again, quadratic age was a non-significant predictor of symptom directionality and was excluded from the GCM. At the within- and between-individual levels, DHEA and testosterone levels were non-significant predictors of symptom directionality. We then tested a GCM that removed age from the model and just DHEA and testosterone were entered simultaneously to examine whether hormones showed an effect on symptom directionality within individuals and across time, not related to age. Controlling for DHEA, we found that at the between-individual level, testosterone significantly predicted symptom directionality in showing males with high morning testosterone level had a preponderance of externalizing symptoms than internalizing symptoms.

3.7 Discussion

Studies focusing on within gender, specifically males, are rare but an important contribution to extant literature on developing psychopathology symptoms in youth. Though gender preponderance of psychopathologies has been found in previous studies (Zahn-Waxler et al., 2006; Zahn-Waxler et al., 2015; Zahn-Waxler et al., 2008), the benefit in diving deeper into investigating male-specific risk factors, though normative, for elevated psychopathology is to find out who those risk groups are and how best to support them (Graber, 2013). Our study targeted a normative sample of male youth and investigated normal developmental processes (aging, puberty, pubertal hormones) to see whether events that all youth experience influence psychopathology symptoms. Our study focused on male children and adolescents and tracked them over time starting from age 6 years as they aged into adolescence to about age 16-18 years.

We examined psychopathology symptoms severity and directionality of externalizing and internalizing symptoms and how puberty and pubertal hormones played a role in elevating or lessening symptoms severity as well as impact symptom directionality.

In the present study, we found that age drove the effect of symptom directionality and that pubertal timing drove the effect of symptoms severity. We also found that morning level DHEA and testosterone had unique effects on symptoms severity and directionality. Below, we first discuss the utility of accelerated longitudinal design in examining symptoms severity and directionality stability and change across development. Second, we dive deeper into different ways of thinking about male-specific developmental trajectories when accounting for age, time, puberty, and pubertal hormones. Third, we discuss the role of pubertal hormones as neurobiological indicators of symptoms severity and directionality in male adolescents. Lastly, we interpret the findings on symptom directionality and how these effects are different from symptoms severity.

This study was a longitudinal design tracking youth across development as they aged and matured, and like many mixed longitudinal designs, there were youth overlapping in age within a cohort of data collection time period (Moerbeek, 2011; Hoffman, 2013). Accelerated longitudinal designs or cohort sequential modeling addresses age overlaps among cohorts and allows for models to capture developmental trajectories of each youth's starting age within each cohort and compare to other youths of similar ages in different cohorts, as illustrated in Figures 3.10.2, 3.10.3, and 3.10.5. We identified "cohort" as YEARS in this study tracking youth's development spanning a little over 6 years. Cohort sequential modeling can further validate developmental trajectory trends among youth at a certain age in one cohort to youth of the same age in a different cohort. This was the first study to utilize cohort sequential modeling to track

children and adolescent symptoms severity and directionality across development and is especially novel in that we focused on male-specific development. Previous studies on symptoms severity and directionality with adolescents generally focused on symptoms as predictors of neurobiological outcomes, such as cortisol diurnal rhythm (Shirtcliff & Essex, 2008; Ruttle, Serbin, Stack, Schwartzman, & Shirtcliff, 2011) or hormone coupling (Marceau et al., 2015). To date, one study examined risk factors of symptoms severity and directionality as outcomes longitudinally with adolescents (Essex et al., 2006); however, in that study, puberty and age were not examined as risk factors for symptoms trajectory. Future research may benefit in using accelerated longitudinal design to study symptoms severity and directionality when considering developmental trajectories over time.

When thinking about child and adolescent development, there are many caveats to consider that explain trajectory trends and particularly in understanding psychopathology symptoms fluctuations over time. At the between-individual level, factors such as age, pubertal timing, pubertal hormones, and the passage of time are all important to consider to capture the full picture of developmental trajectories in youth. Psychopathology symptoms may get overshadowed by what is considered normative changes when not careful to account for these normal developmental factors. This may be especially true for adolescent males who have elevated symptoms severity as they undergo puberty. In the present study, symptoms severity was driven by pubertal timing in male adolescents; specifically, early developers had greater symptoms severity than average and late developers. Pubertal timing was not the only explanatory factor of symptoms severity between individuals; age at which youth were observed at the start of this study also explained elevated symptoms severity. Elevated symptoms severity was particularly shown for youth who were older at Time 1; however, the oldest youth within the

first time point had less symptoms severity with age. Within-individual stability of symptoms severity was found in the overall sample in which the passage of time predicted decreasing symptoms. Furthermore, fluctuations in symptoms severity varied by youths' starting age at Time 1, which accounted for elevation of symptoms in some youth as they aged. Altogether, findings from this study further showed the importance of accounting for developmental factors when examining symptoms severity over time.

Development is complex, but trajectory trends in symptoms severity can be detected as was observed in this study. Age was found as a developmental indicator of symptoms severity, and pubertal timing and pubertal hormones also contributed to explaining symptoms severity. Findings from this study suggested that youth getting older did not simply inform symptoms severity in those who were aging and maturing chronologically but that physical and neurobiological development played a role as well. The concept of "raging hormones" has been misconstrued as a negative consequence of pubertal maturation (Buchanan, Eccles, & Becker, 1992; Dahl, 2003; Shirtcliff, 2009), and this misperception may overshadow underlying problems facing some adolescent males who have elevated symptoms severity. If hormones unilaterally impact behaviors, one can expect for both high levels of DHEA and testosterone – tertiary hormones responsible for pubertal maturation through the adrenal and gonadal glands – to predict elevated symptoms severity. The present study found DHEA and testosterone showed differing functions related to symptoms severity where DHEA predicted lower symptoms, and testosterone predicted elevated symptoms at the within-individual level. This finding provided evidence that pubertal hormones each had unique effects on symptoms severity, and the finding on testosterone's effect on symptoms severity during adolescence was in line with previous extant literature (see systematic review: Duke, Balzer, & Steinbeck, 2014).

Previous longitudinal research on psychopathology symptoms during adolescence generally focused on specific psychopathologies, and in recent years, studies have begun to examine longitudinal heterotypic comorbidity of symptoms in adolescents (Jones et al., 2018; Laceulle, Chung, Vollebergh, & Ormel, 2020; O'Reilly et al., 2020). Despite these emerging works, there is still a dearth of longitudinal studies on symptoms severity and directionality as youth transition through puberty. In this study, symptom directionality was driven by the effect of youth's age. Unlike the findings on symptoms severity, time did not predict directionality, which meant youths maintained symptom type over time. Youths who started with more internalizing symptoms consistently showed stability in internalizing symptoms and vice versa for youth who started with more externalizing symptoms. Pubertal timing did not have an effect on symptom directionality one way or another and instead was a suppressor of the effect of age on symptom directionality confirming that age had a robust effect on symptom directionality. In the overall sample, male youths had a preponderance of externalizing symptoms; however, symptom domain switched for youths who were older than other youths at Time 1. Older youths at Time 1 showed a preponderance of internalizing symptoms over time. There is a dearth of studies on male-specific internalizing symptoms preponderance; however, one study found that social connectedness predicted depressive symptoms mediated by loneliness in older male adolescents (Jose & Lim, 2014). While in that study researchers found more social connectedness leading to reduced loneliness which in turn reduced depressive symptoms for older male adolescents, less social connectedness and higher loneliness could potentially lead to higher depressive symptoms. More research is needed to investigate risk factors that lead to elevated internalizing symptoms in older adolescent males as they develop over time.

This was the first study to date to examine within males only sample of youth longitudinally as they underwent puberty and tested pubertal hormonal effects on psychopathology symptoms severity and directionality. Elevated testosterone levels during puberty is considered normative, especially in male adolescents (Khairullah et al., 2014; Vandewalle et al., 2014). In typical development, as puberty has already undergone, testosterone levels increase, which speeds up physical maturation. In the present study, we examined whether elevated testosterone predicted symptom directionality. Results from the GCM of testosterone as a predictor on symptoms directionality over time showed a preponderance of externalizing symptoms. Elevated testosterone has also been found to be associated with externalizing symptoms in other studies (Mundy et al., 2015; Archer, 2006; Grotzinger, Mann, et al., 2018). The testosterone and externalizing link can also be ascribed to individual differences such as those who have a tendency to be more dominant (Carré & Archer, 2018). Whether male adolescents in this study fit that characteristic to predict externalizing symptoms due to high testosterone levels is yet to be determined but is a potential explanation for this association. More studies are needed with the attention on adolescent males transitioning through puberty, the role of pubertal hormones, and monitoring development of psychopathology symptoms onset, stability, and change.

3.8 Limitations

Some study strengths and limitations are noted. First, a limitation of this data is that it is not a true longitudinal design but instead a mixed longitudinal design. A strength of the present study is the use of accelerated longitudinal design addressed this limitation by allowing researchers to account for participant attrition, funding budget, and other study factors that limit data collection with the same number of participants in each cohort and same observational data.

Another strength of this study was the combination of cohort sequential modeling with growth curve models to examine developmental changes in psychopathology symptoms within- and between-individual fluctuations over time. Another strength of this study was that measures of puberty and pubertal hormones were included alongside developmental age in the growth curve models to test neurobiological effect on psychopathology symptoms. A limitation was that puberty and pubertal hormones were not measured across all time points, which disallowed us to investigate pubertal tempo (rate of change in physical maturation over time across developmental age). Investigation of pubertal tempo and its association with psychopathology symptoms severity/directionality may be a mechanistic key to better understanding symptoms comorbidity in male adolescents. Future research can fill this knowledge gap. Females were excluded, and gender differences were not tested, which was beyond the scope of the present study. Future research may consider a study that examine within females only and then test gender differences as a means to examine sex as a biological factor on psychopathology symptoms severity and directionality over time. The symptoms severity and directionality methodological approach allowed us to investigate heterotypic comorbidity but limits from examining homotypic comorbidity (elevated multiple psychopathology symptoms in one domain). For example, an examination of elevated depressive symptoms along with anxiety symptoms, both of which fall in the internalizing domain. An investigation of homotypic comorbidity and symptom severity across development in males has yet to be studied. Future research can also fill this knowledge gap in the literature.

References

Ablow, J. C., Measelle, J. R., Kraemer, H. C., Harrington, R., Luby, J., Smider, N., … Kupper, D. J. (1999). The MacArthur Three-City Outcome Study: Evaluating Multi-Informant Measures of Young Children's Symptomatology. *Journal of the American Academy of Child & Adolescent Psychiatry*, *38*(12), 1580–1590. https://doi.org/10.1097/00004583-199912000-00020

Afzali, M. H., Sunderland, M., Carragher, N., & Conrod, P. (2018). The Structure of Psychopathology in Early Adolescence: Study of a Canadian Sample. *The Canadian Journal of Psychiatry*. https://doi.org/10.1177/0706743717737032

Archer, J. (2006). Testosterone and human aggression: An evaluation of the challenge hypothesis. *Neuroscience and Biobehavioral Reviews*, *30*(3), 319–345. https://doi.org/10.1016/j.neubiorev.2004.12.007

Blackburn-Munro, G., & Blackburn-Munro, R. E. (2001). Chronic pain, chronic stress and depression: Coincidence or consequence? *Journal of Neuroendocrinology*. https://doi.org/10.1046/j.0007-1331.2001.00727.x

Black, S. R., Lerner, M. D., Shirtcliff, E. A., & Klein, D. N. (2018). Patterns of neuroendocrine coupling in 9-year-old children: Effects of sex, body-mass index, and life stress. *Biological Psychology*, *132*, 252–259. https://doi.org/10.1016/J.BIOPSYCHO.2017.11.004

Buchanan, C. M., Eccles, J. S., & Becker, J. B. (1992). Are adolescents the victims of raging hormones? Evidence for activational effects of hormones on moods and behavior at adolescence. *Psychological Bulletin*. https://doi.org/10.1037/0033-2909.111.1.62

Burke, J. D., Loeber, R., Lahey, B. B., & Rathouz, P. J. (2005). Developmental transitions among affective and behavioral disorders in adolescent boys. *Journal of Child Psychology and Psychiatry and Allied Disciplines*. https://doi.org/10.1111/j.1469-7610.2005.00422.x

Byrne, M. L., Whittle, S., Vijayakumar, N., Dennison, M., Simmons, J. G., & Allen, N. B. (2017). A systematic review of adrenarche as a sensitive period in neurobiological development and mental health. *Developmental Cognitive Neuroscience*, *25*, 12–28. https://doi.org/10.1016/J.DCN.2016.12.004

Carragher, N., Teesson, M., Sunderland, M., Newton, N. C., Krueger, R. F., Conrod, P. J., … Slade, T. (2016). The structure of adolescent psychopathology: A symptom-level analysis. *Psychological Medicine*. https://doi.org/10.1017/S0033291715002470

Carré, J. M., & Archer, J. (2018). Testosterone and human behavior: the role of individual and contextual variables. *Current Opinion in Psychology*. https://doi.org/10.1016/j.copsyc.2017.03.021

Coleman, L., & Coleman, J. (2002). The measurement of puberty: a review. *Journal of Adolescence*, *25*(5), 535–550. https://doi.org/10.1006/JADO.2002.0494

Dahl, R. (2003). Beyond Raging Hormones : The Tinderbox in the Teenage Brain. *Cerebrum: The Dana Forum on Brain Science*.

Deardorff, J., Hoyt, L. T., Carter, R., & Shirtcliff, E. A. (2019). Next Steps in Puberty Research: Broadening the Lens Toward Understudied Populations. *Journal of Research on Adolescence*. https://doi.org/10.1111/jora.12402

Doerfler, L. A., Felner, R. D., Rowlison, R. T., Raley, P. A., & Evans, E. (1988). Depression in children and adolescents: A comparative analysis of the utility and construct validity of two assessment measures. *Journal of Consulting and Clinical Psychology*, *56*(5), 769–772. https://doi.org/10.1037/0022-006X.56.5.769

Duke, S. A., Balzer, B. W. R., & Steinbeck, K. S. (2014). Testosterone and Its Effects on Human Male Adolescent Mood and Behavior: A Systematic Review. *Journal of Adolescent Health*, *55*(3), 315–322. https://doi.org/10.1016/j.jadohealth.2014.05.007

Essex, M. J., Kraemer, H. C., Armstrong, J. M., Boyce, W. T., Goldsmith, H. H., Klein, M. H., … Kupfer, D. J. (2006). Exploring risk factors for the emergence of children's mental health problems. *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.63.11.1246

Fishbain, D. A., Cutler, R., Rosomoff, H. L., & Rosomoff, R. S. (1997). Chronic pain-associated depression: Antecedent or consequence of chronic pain? A review. *Clinical Journal of Pain*. https://doi.org/10.1097/00002508-199706000-00006

Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.177.6.499

Graber, J. A. (2013). Pubertal timing and the development of psychopathology in adolescence and beyond. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2013.04.003

Granger, D. A., Shirtcliff, E. A., Zahn-Waxler, C., Usher, B., Klimes-Dougan, B., & Hastings, P. (2003). Salivary testosterone diurnal variation and psychopathology in adolescent males and females: Individual differences and developmental effects. *Development and Psychopathology*. https://doi.org/10.1017/S0954579403000233

Grotzinger, A. D., Mann, F. D., Patterson, M. W., Tackett, J. L., Tucker-Drob, E. M., & Harden, K. P. (2018). Hair and Salivary Testosterone, Hair Cortisol, and Externalizing Behaviors in Adolescents. *Psychological Science*, *29*(5), 688–699. https://doi.org/10.1177/0956797617742981

Guran, T., Firat, I., Yildiz, F., Kaplan Bulut, I., Dogru, M., & Bereket, A. (2015). Reference values for serum dehydroepiandrosterone-sulphate in healthy children and adolescents with emphasis on the age of adrenarche and pubarche. *Clinical Endocrinology*. https://doi.org/10.1111/cen.12612

Havelock, J. C., Auchus, R. J., & Rainey, W. E. (2004). The rise in adrenal androgen biosynthesis: Adrenarche. *Seminars in Reproductive Medicine*. https://doi.org/10.1055/s-2004-861550

Herman-Giddens, M. E., Wang, L., & Koch, G. (2001). Secondary Sexual Characteristics in Boys. *Archives of Pediatrics & Adolescent Medicine*, *155*(9), 1022. https://doi.org/10.1001/archpedi.155.9.1022

Hodges, K. (1990). Depression and anxiety in children: A comparison of self-report questionnaires to clinical interview. *Psychological Assessment*, *2*(4), 376–381. https://doi.org/10.1037/1040-3590.2.4.376

Hoffman, L. (2013). Longitudinal Analysis: Modeling Within-Person Fluctuation and Change. In *Journal of Chemical Information and Modeling*. https://doi.org/10.1017/CBO9781107415324.004

Jones, H. J., Heron, J., Hammerton, G., Stochl, J., Jones, P. B., Cannon, M., … Zammit, S. (2018). Investigating the genetic architecture of general and specific psychopathology in adolescence. *Translational Psychiatry*, *8*(1), 145. https://doi.org/10.1038/s41398-018-0204-9

Jose, P. E., & Lim, B. T. L. (2014). Social Connectedness Predicts Lower Loneliness and Depressive Symptoms over Time in Adolescents. *Open Journal of Depression*, *03*(04), 154–163. https://doi.org/10.4236/ojd.2014.34019

Kessler, R. C. (2013). The effects of stressful life events on depression. In *Depression: The Science of Mental Health* (Vol. 6, pp. 67–91). https://doi.org/10.1146/annurev.psych.48.1.191

Khairullah, A., Cousino Klein, L., Ingle, S. M., May, M. T., Whetzel, C. A., Susman, E. J., & Paus, T. (2014). Testosterone Trajectories and Reference Ranges in a Large Longitudinal Sample of Male Adolescents. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0108838

Khesht-Masjedi, M., Shokrgozar, S., Abdollahi, E., Golshahi, M., & Sharif-Ghaziani, Z. (2017). Comparing depressive symptoms in teenage boys and girls. *Journal of Family Medicine and Primary Care*. https://doi.org/10.4103/jfmpc.jfmpc_129_17

Kovacs, M. (2010). *CDI 2 Children's Depression Inventory 2* (2nd ed.). Retrieved from https://www.pearsonassessments.com/store/usassessments/en/Store/Professional-Assessments/Personality-6-Biopsychosocial/Children7s-Depression-Inventory-2/p/100000636.html

Kraemer, H. C., Measelle, J. R., Ablow, J. C., Essex, M. J., Boyce, W. T., & Kupfer, D. J. (2003). A new approach to integrating data from multiple informants in psychiatric assessment and research: Mixing and matching contexts and perspectives. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.160.9.1566

Kushki, A., Anagnostou, E., Hammill, C., Duez, P., Brian, J., Iaboni, A., … Lerch, J. P. (2019). Examining overlap and homogeneity in ASD, ADHD, and OCD: a data-driven, diagnosis-agnostic approach. *Translational Psychiatry*, *9*(1), 318. https://doi.org/10.1038/s41398-019-0631-2

Laceulle, O. M., Chung, J. M., Vollebergh, W. A. M., & Ormel, J. (2020). The wide-ranging life outcome correlates of a general psychopathology factor in adolescent psychopathology. *Personality and Mental Health*. https://doi.org/10.1002/pmh.1465

Lahey, B. B., Rathouz, P. J., Van Hulle, C., Urbano, R. C., Krueger, R. F., Applegate, B., … Waldman, I. D. (2008). Testing Structural Models of DSM-IV Symptoms of Common Forms of Child and Adolescent Psychopathology. *Journal of Abnormal Child Psychology*, *36*(2), 187–206. https://doi.org/10.1007/s10802-007-9169-5

Lemery-Chalfant, K., Schreiber, J. E., Schmidt, N. L., Van Hulle, C. A., Essex, M. J., & Goldsmith, H. H. (2007). Assessing Internalizing, Externalizing, and Attention Problems in Young Children: Validation of the MacArthur HBQ. *Journal of the American Academy of Child & Adolescent Psychiatry*, *46*(10), 1315–1323. https://doi.org/10.1097/chi.0b013e3180f616c6

López-Villalobos, J. A., Andrés-De Llano, J. M., Rodríguez-Molinero, L., Garrido-Redondo, M., Sacristán-Martín, A. M., Martínez-Rivera, M. T., … Sánchez-Azón, M. I. (2014). Prevalence of oppositional defiant disorder in Spain. *Revista de Psiquiatría y Salud Mental (English Edition)*. https://doi.org/10.1016/j.rpsmen.2014.02.006

Maras, A., Laucht, M., Gerdes, D., Wilhelm, C., Lewicka, S., Haack, D., … Schmidt, M. H. (2003). Association of testosterone and dihydrotestosterone with externalizing behavior in adolescent boys and girls. *Psychoneuroendocrinology*. https://doi.org/10.1016/S0306-4530(02)00119-1

Marceau, K., Ram, N., Houts, R. M., Grimm, K. J., & Susman, E. J. (2011). Individual differences in boys' and girls' timing and tempo of puberty: modeling development with nonlinear growth models. *Developmental Psychology*, *47*(5), 1389–1409. https://doi.org/10.1037/a0023838

Marceau, K., Ruttle, P. L., Shirtcliff, E. A., Hastings, P. D., Klimes-Dougan, B., & Zahn-Waxler, C. (2015). Within-person coupling of changes in cortisol, testosterone, and DHEA across the day in adolescents. *Developmental Psychobiology*. https://doi.org/10.1002/dev.21173

March, J S. (2004). Multidimensional Anxiety Scale for Children (MASC^{TM}). *The Use of Psychological Testing for Treatment Planning and Outcomes Assessment: Instruments for Children and Adolescents*.

March, John S., Parker, J. D. A., Sullivan, K., Stallings, P., & Conners, C. K. (1997). The Multidimensional Anxiety Scale for Children (MASC): Factor structure, reliability, and validity. *Journal of the American Academy of Child and Adolescent Psychiatry*. https://doi.org/10.1097/00004583-199704000-00019

March, John S., Sullivan, K., & Parker, J. (1999). Test-retest reliability of the Multidimensional Anxiety Scale for Children. *Journal of Anxiety Disorders*. https://doi.org/10.1016/S0887-6185(99)00009-2

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood*, *45*(239), 13–23. https://doi.org/10.1136/ADC.45.239.13

McCabe, K. M., Rodgers, C., Yeh, M., & Hough, R. (2004). Gender differences in childhood onset conduct disorder. *Development and Psychopathology*, *16*(1), 179–192. https://doi.org/10.1017/S0954579404044463

McLaughlin, K. A., & King, K. (2014). Developmental Trajectories of Anxiety and Depression in Early Adolescence. *Journal of Abnormal Child Psychology*. https://doi.org/10.1007/s10802-014-9898-1

Mendle, J., Beltz, A. M., Carter, R., & Dorn, L. D. (2019). Understanding Puberty and Its Measurement: Ideas for Research in a New Generation. *Journal of Research on Adolescence*. https://doi.org/10.1111/jora.12371

Mendle, J., & Ferrero, J. (2012). Detrimental psychological outcomes associated with pubertal timing in adolescent boys. *Developmental Review*, *32*(1), 49–66. https://doi.org/10.1016/J.DR.2011.11.001

Mendle, J., Turkheimer, E., & Emery, R. E. (2007). Detrimental psychological outcomes associated with early pubertal timing in adolescent girls. *Developmental Review*. https://doi.org/10.1016/j.dr.2006.11.001

Moerbeek, M. (2011). The effects of the number of cohorts, degree of overlap among cohorts, and frequency of observation on power in accelerated longitudinal designs. *Methodology*. https://doi.org/10.1027/1614-2241/a000019

Morken, G., Lilleeng, S., & Linaker, O. M. (2002). Seasonal variation in suicides and in admissions to hospital for mania and depression. *Journal of Affective Disorders*, *69*(1–3), 39–45. https://doi.org/10.1016/S0165-0327(00)00373-6

Morris, N. M., & Udry, J. R. (1980). Validation of a self-administered instrument to assess stage of adolescent development. *Journal of Youth and Adolescence*, *9*(3), 271–280. https://doi.org/10.1007/BF02088471

Mundy, L. K., Romaniuk, H., Canterford, L., Hearps, S., Viner, R. M., Bayer, J. K., … Patton, G. C. (2015). Adrenarche and the emotional and behavioral problems of late childhood. *Journal of Adolescent Health*. https://doi.org/10.1016/j.jadohealth.2015.09.001

Norris, S. A., & Richter, L. M. (2005). Usefulness and Reliability of Tanner Pubertal Self-Rating to Urban Black Adolescents in South Africa. *Journal of Research on Adolescence*, *15*(4), 609–624. https://doi.org/10.1111/j.1532-7795.2005.00113.x

O'Reilly, L. M., Pettersson, E., Quinn, P. D., Klonsky, E. D., Lundström, S., Larsson, H., … D'Onofrio, B. M. (2020). The Association Between General Childhood Psychopathology and Adolescent Suicide Attempt and Self-Harm: A Prospective, Population-Based Twin Study. *Journal of Abnormal Psychology*. https://doi.org/10.1037/abn0000512

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence*, *17*(2), 117–133. https://doi.org/10.1007/BF01537962

Rabbani, A., Noorian, S., Fallah, J. S., Setoudeh, A., Sayarifard, F., & Abbasi, F. (2013). Reliability of pubertal self assessment method: an Iranian study. *Iranian Journal of Pediatrics*, *23*(3), 327–332. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23795257

Raudenbush, S.W., Bryk, A.S., & Congdon, R. (2019). *HLM 8 for Windows [Computer software]*. Skokie, IL: Scientific Software International, Inc.

Rucklidge, J. J. (2010, June). Gender Differences in Attention-Deficit/Hyperactivity Disorder. *Psychiatric Clinics of North America*, Vol. 33, pp. 357–373. https://doi.org/10.1016/j.psc.2010.01.006

Ruttle, P. L., Serbin, L. A., Stack, D. M., Schwartzman, A. E., & Shirtcliff, E. A. (2011). Adrenocortical attunement in mother-child dyads: Importance of situational and behavioral characteristics. *Biological Psychology*, *88*(1), 104–111. https://doi.org/10.1016/j.biopsycho.2011.06.014

Schmidt, N. L., Van Hulle, C. A., Brooker, R. J., Meyer, L. R., Lemery-Chalfant, K., & Goldsmith, H. H. (2013). Wisconsin Twin Research: Early Development, Childhood Psychopathology, Autism, and Sensory Over-responsivity. *Twin Research and Human Genetics*, *16*(1), 376–384. https://doi.org/10.1017/thg.2012.105

Sher, L., Siever, L. J., Goodman, M., McNamara, M., Hazlett, E. A., Koenigsberg, H. W., & New, A. S. (2015). Gender differences in the clinical characteristics and psychiatric comorbidity in patients with antisocial personality disorder. *Psychiatry Research*, *229*(3), 685–689. https://doi.org/10.1016/j.psychres.2015.08.022

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology*. https://doi.org/10.1002/dev.20336

Sizonenko, P. C., & Paunier, L. (1975). Hormonal Changes in Puberty III: Correlation of Plasma Dehydroepiandrosterone,Testosterone, FSH, and LH with Stages of Puberty and Bone Age in Normal Boys and Girls and in Patients with Addison's Disease or Hypogonadism or with Premature or Late Adrenarche. *The Journal of Clinical Endocrinology & Metabolism*, *41*(5), 894–904. https://doi.org/10.1210/jcem-41-5-894

Tanner, J. M. (1962). Growth at adolescence. In Oliver and Boyd (Ed.), *The Eugenics Review* (2nd ed., Vol. 54). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982342/

Timbremont, B., Braet, C., & Dreessen, L. (2004). Assessing Depression in Youth: Relation Between the Children's Depression Inventory and a Structured Interview. *Journal of Clinical Child & Adolescent Psychology*, *33*(1), 149–157. https://doi.org/10.1207/S15374424JCCP3301_14

Whittle, S., Simmons, J. G., Byrne, M. L., Strikwerda-Brown, C., Kerestes, R., Seal, M. L., … Allen, N. B. (2014). Associations between early adrenarche, affective brain function and mental health in children. *Social Cognitive and Affective Neuroscience*. https://doi.org/10.1093/scan/nsv014

Willett, J. B., Singer, J. D., & Martin, N. C. (1998). The design and analysis of longitudinal studies of development and psychopathology in context: Statistical models and methodological recommendations. *Development and Psychopathology*. https://doi.org/10.1017/S0954579498001667

Vandewalle, S., Taes, Y., Fiers, T., Toye, K., Van Caenegem, E., Roggen, I., … Kaufman, J. M. (2014). Associations of sex steroids with bone maturation, bone mineral density, bone geometry, and body composition: A cross-sectional study in healthy male adolescents. *Journal of Clinical Endocrinology and Metabolism*. https://doi.org/10.1210/jc.2013-3887

Van Hulle, C. A., Moore, M. N., Shirtcliff, E. A., Lemery-Chalfant, K., & Goldsmith, H. H. (2015). Genetic and Environmental Contributions to Covariation Between DHEA and Testosterone in Adolescent Twins. *Behavior Genetics*, *45*(3), 324–340. https://doi.org/10.1007/s10519-015-9709-7

Zahn-Waxler, C., Crick, N. R., Shirtcliff, E. A., & Woods, K. E. (2006). *Developmental Psychopathology*. John Wiley & Sons, Inc.

Zahn-Waxler, C., Crick, N. R., Shirtcliff, E. A., & Woods, K. E. (2015). The Origins and Development of Psychopathology in Females and Males. In *Developmental Psychopathology* (pp. 76–138). https://doi.org/10.1002/9780470939383.ch4

Zahn-Waxler, C., Shirtcliff, E. A., & Marceau, K. (2008). Disorders of Childhood and Adolescence: Gender and Psychopathology. *Annual Review of Clinical Psychology*, *4*(1), 275–303. https://doi.org/10.1146/annurev.clinpsy.3.022806.091358

3.9 Tables

Table 3.9.1 Psychopathology Measures Means and Standard Deviations Statistics

Psychopathology measure	Informant	Mean(SD)			Range		
		T1	T2	T3	T1	T2	T3
Child Depression Inventory	Mother	0.151(0.18)	0.517(0.37)	-	0-1.04	0-2.53	-
	Father	0.127(0.16)	0.559(0.34)	-	0-1.08	0-2.00	-
Multidimensional Anxiety Scale for Children	Mother	-	1.123(0.39)	-	-	0.05-2.31	-
	Youth	-	-	0.979(0.37)	-	-	0-2.36
Health and Behavior Questionnaire, Symptoms Scale							
internalizing	Mother	0.327(0.24)	13.04(10.60)	0.188(0.20)	0-1.52	0-67	0-1.22
	Youth	-	2.37(0.58)	2.297(0.62)	-	1-3.97	1-4.40
externalizing	Mother	0.344(0.28)	7.85(7.46)	0.177(0.211)	0-1.80	0-46	0-1.12
	Youth	-	2.219(0.63)	2.146(0.64)	-	1-5.00	1-5.32

Table 3.9.2 Principal Component Analysis Factor Loadings for Symptoms Severity and Directionality

	Time 1		Time 2		Time 3	
	Severity	Directionality	Severity	Directionality	Severity	Directionality
depression	0.689	0.461	0.799	0.365	0.563	0.732
anxiety	0.579	0.687	0.587	0.708	0.536	0.752
conduct disorder	0.847	-0.311	0.805	-0.329	0.831	-0.304
oppositional defiant disorder	0.870	-0.192	0.864	-0.155	0.805	-0.360
overt aggression	0.845	-0.338	0.702	-0.440	0.724	-0.376

Table 3.9.3 Predictors of Symptom Severity

3.9.3.a Symptom severity base model

	B	SE	t	df	p
intercept	5.001	0.024	207.39	1834	<0.001
years	-0.012	0.006	-1.86	1834	0.063

3.9.3.b Age predicted symptom severity

Level 2 predictors	B	SE	t	df	p
intercept	4.986	0.03	180.15	1580	<0.001
age	0.052	0.04	1.50	1580	0.134
years	-0.022	0.01	-3.05	1580	0.002
age	0.011	0.01	1.44	1580	0.150

3.9.3.c Age and quadratic age predicted symptom severity

Level 2 predictors	B	SE	t	df	p
intercept	4.981	0.03	181.71	1579	<0.001
age	0.151	0.06	2.71	1579	0.007
quadratic age	-0.038	0.02	-2.09	1579	0.037
years	-0.021	0.01	-2.95	1579	0.003
age	-0.009	0.01	-0.66	1579	0.507
quadratic age	0.007	0.01	1.46	1579	0.144

3.9.3.d Pubertal timing predicted symptom severity

Level 2 predictors	B	SE	t	df	p
intercept	5.143	0.06	84.11	453	<0.001
age	0.195	0.12	1.57	453	0.117
quadratic age	-0.066	0.04	-1.52	453	0.129
pubertal timing	0.176	0.06	2.87	453	0.004
years	-0.035	0.01	-3.63	453	<0.001
age	-0.010	0.02	-0.54	453	0.593
quadratic age	0.009	0.01	1.37	453	0.173
pubertal timing	-0.013	0.01	-1.34	453	0.181

3.9.3.e DHEA and testosterone predicted symptom severity

Level 2 predictors	B	SE	t	df	p
intercept	5.125	0.080	62.24	302	<0.001
age	0.002	0.070	0.03	302	0.978
DHEA	-0.194	0.090	-2.16	302	0.031
testosterone	0.355	0.140	2.63	302	0.009
years	-0.033	0.010	-2.83	302	0.005
age	0.007	0.010	0.71	302	0.476
DHEA	0.024	0.010	1.72	302	0.086
testosterone	-0.039	0.020	-1.77	302	0.078

Table 3.9.4 Predictors of Symptom Directionality

3.9.4.a Symptom directionality base model

	B	SE	t	df	p
intercept	-0.000	0.02	-0.01	1834	0.990
years	0.003	0.01	0.51	1834	0.612

3.9.4.b Age predicted symptom directionality

Level 2 predictors	B	SE	t	df	p
intercept	-0.049	0.03	-1.73	1580	0.085
age	0.068	0.03	2.27	1580	0.023
years	0.006	0.01	0.75	1580	0.456
age	-0.005	0.01	-0.72	1580	0.470

3.9.4.c Age not pubertal timing predicted symptom directionality

Level 2 predictors	B	SE	t	df	p
intercept	0.019	0.06	0.34	456	0.731
years	0.006	0.01	0.53	454	0.598
age	-0.015	0.01	-2.04	454	0.042
pubertal timing	-0.010	0.01	-1.11	454	0.267

3.9.4.d DHEA and testosterone (net of age) did not predict symptom directionality

Level 2 predictors	B	SE	t	df	p
intercept	0.011	0.08	0.14	302	0.891
age	0.027	0.09	0.29	302	0.770
DHEA	-0.097	0.10	-0.97	302	0.333
testosterone	0.144	0.15	0.98	302	0.326
years	0.003	0.01	0.22	302	0.824
age	-0.010	0.02	-0.64	302	0.525
DHEA	0.015	0.02	0.95	302	0.341
testosterone	-0.039	0.02	-1.59	302	0.113

3.9.4.e Testosterone predicted symptom directionality

Level 2 predictors	B	SE	t	df	p
intercept	0.018	0.07	0.26	303	0.796
DHEA	-0.097	0.10	-0.97	303	0.331
testosterone	0.157	0.13	1.18	303	0.241
years	-0.002	0.01	-0.15	303	0.884
DHEA	0.016	0.02	0.98	303	0.327
testosterone	-0.045	0.02	-2.03	303	0.044

3.10 Figures

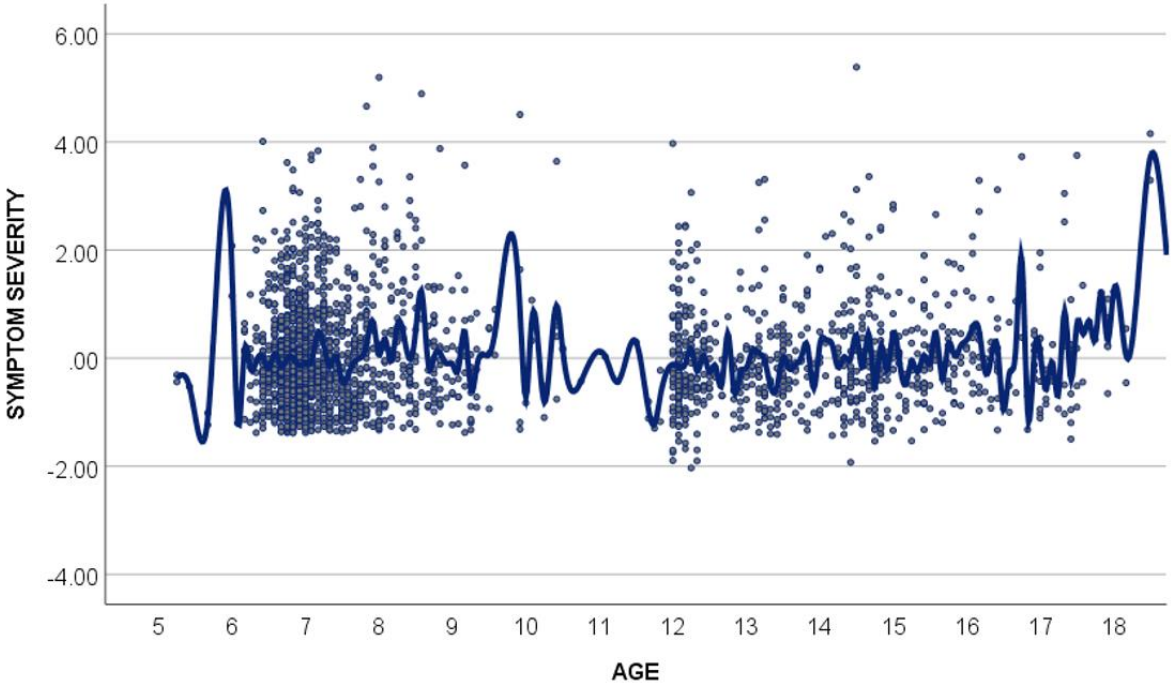

Figure 3.10.1 *A scatterplot of symptom severity across developmental age trajectory. Symptom severity scores are standardized.*

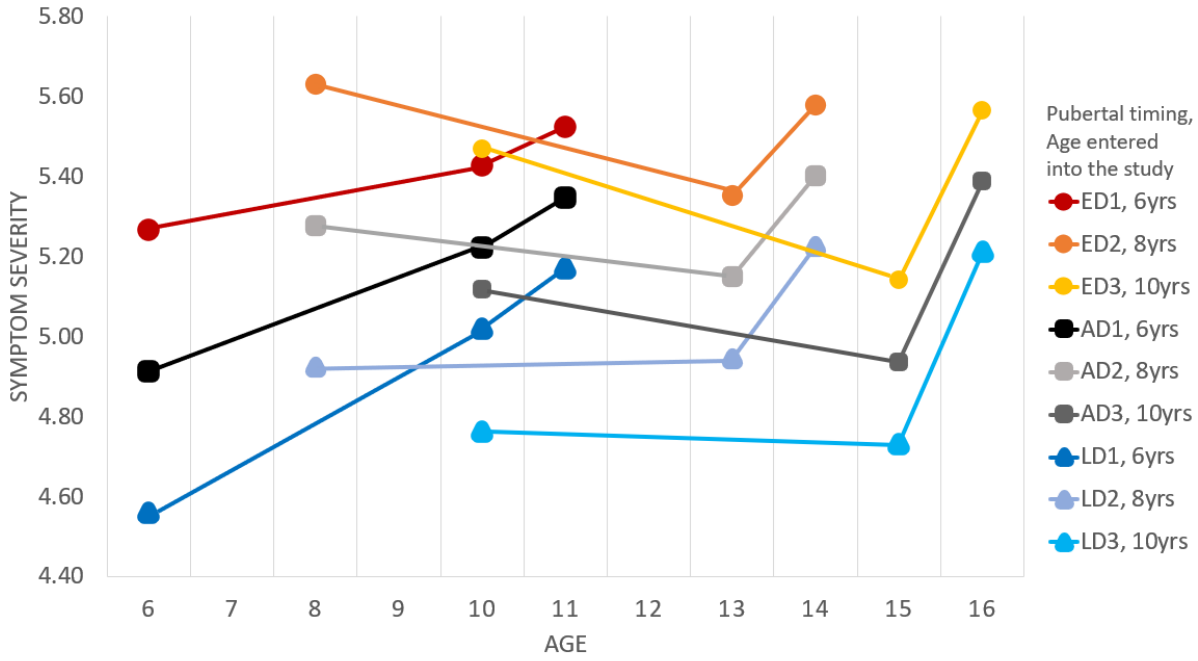

Figure 3.10.2 *Psychopathology symptom severity across development. Lines represent an individual who are late developer (LD), early developer (ED), or average developer (AD) and the age at which they entered the study at either 6, 8, or 10 years old.*

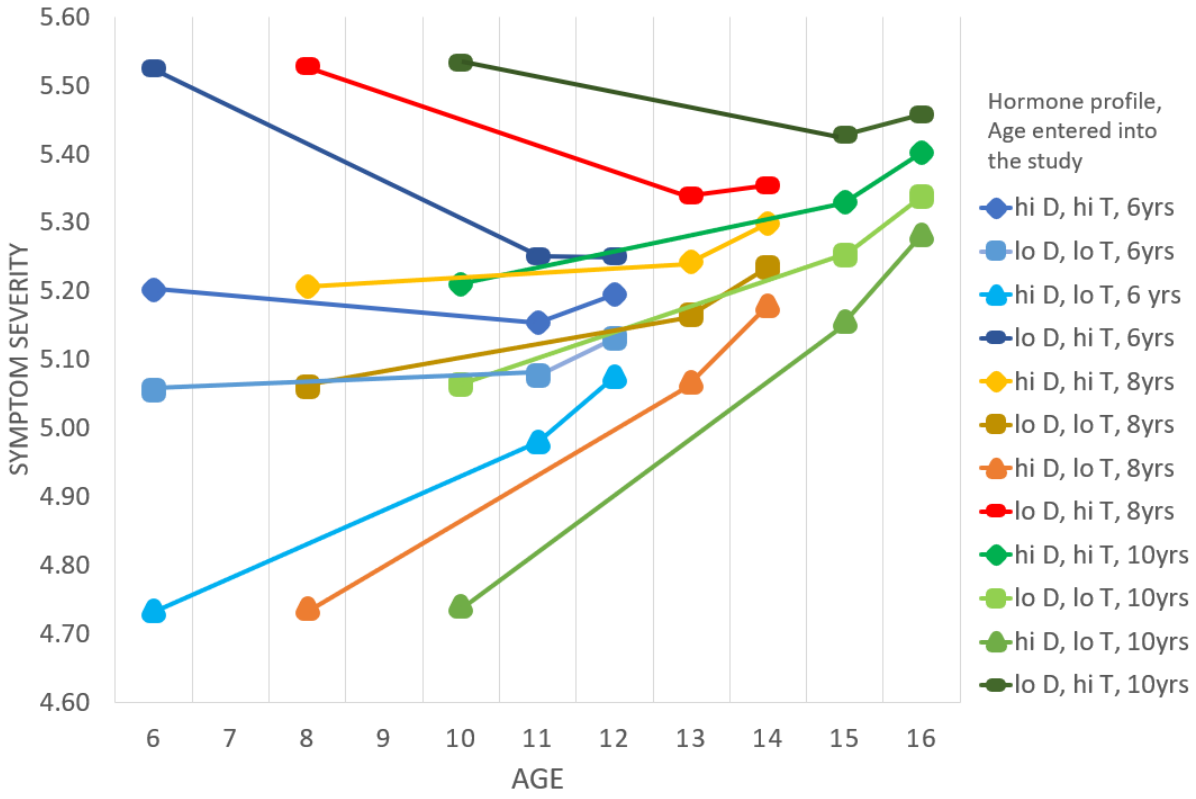

Figure 3.10.3 *DHEA and testosterone effects on psychopathology symptom severity. Hi D = high DHEA, Hi T = high testosterone, Lo D = low DHEA, Lo T = low testosterone.*

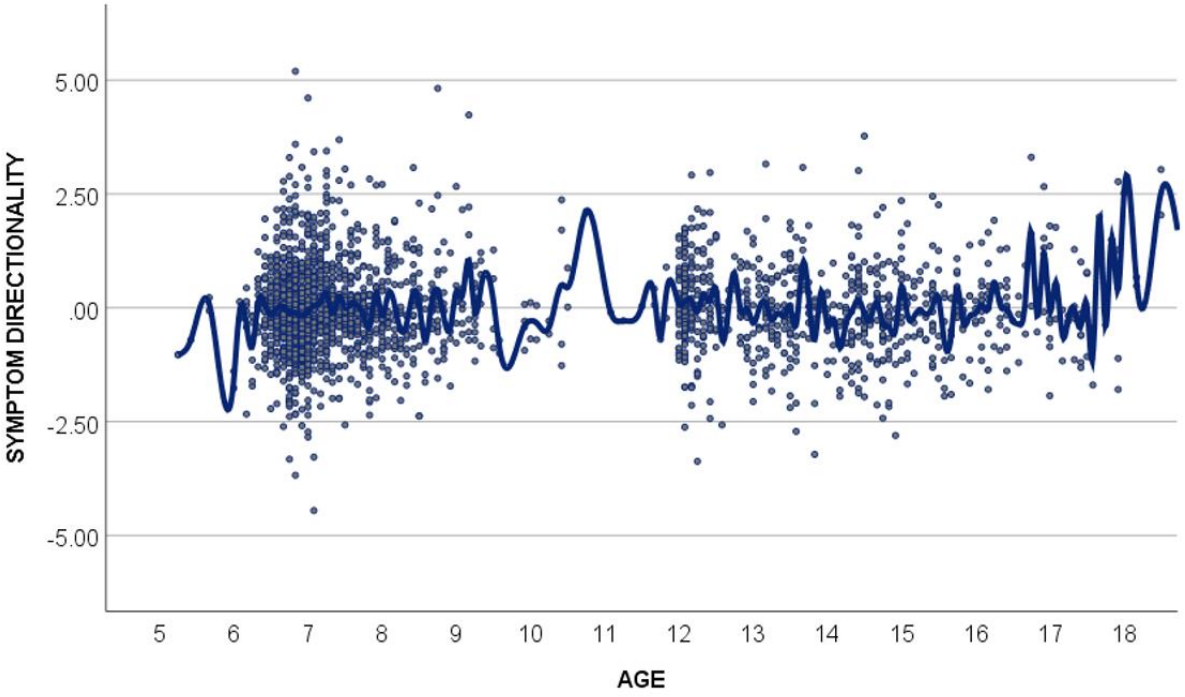

Figure 3.10.4 *Psychopathology symptom directionality across developmental age. Lines below zero mean participants had a preponderance of externalizing domain, and above the zero, participants had a preponderance of internalizing domain.*

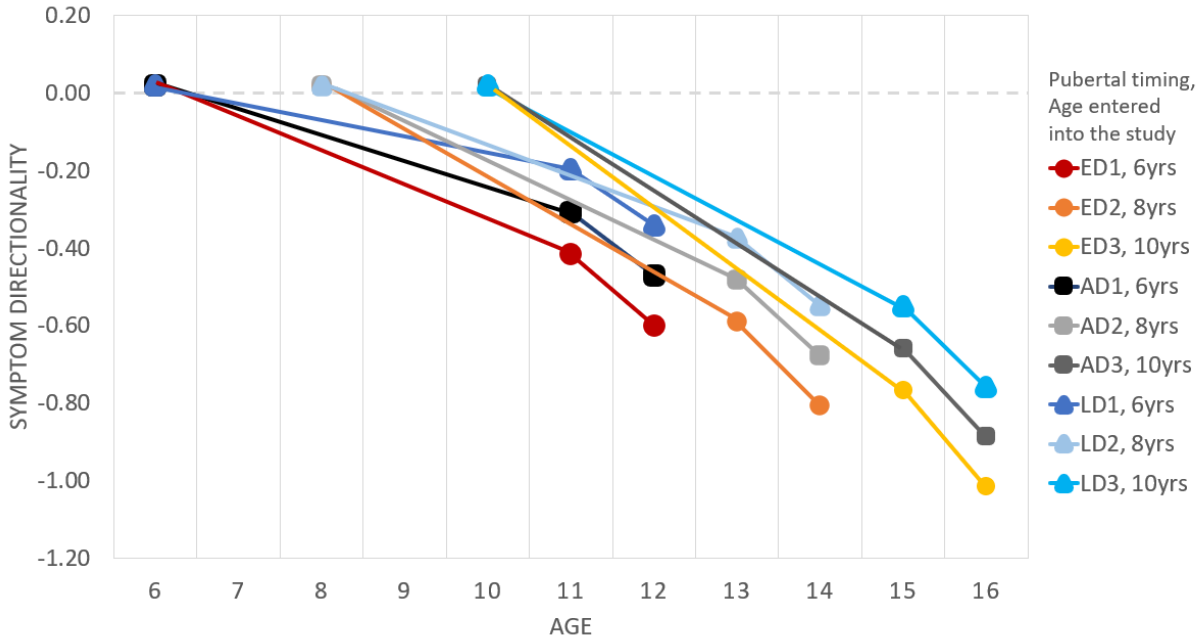

Figure 3.10.5 *Psychopathology symptom directionality of internalizing and externalizing symptoms across development. Positive deviations from the dotted line represent internalizing symptoms, and negative deviations from the dotted line represent externalizing symptoms.*

CHAPTER 4. REPETITIVE BEHAVIORS AND PUBERTAL STATUS PREDICTS PSYCHOPATHOLOGY SYMPTOMS IN ADOLESCENTS WITH AND WITHOUT AUTISM

Jenny M. Phan,[1] Carla Mazefsky,[2] & Elizabeth A. Shirtcliff[1]

Modified from a manuscript to be submitted in journal *Autism.*

4.1 Author Contributions

Jenny Phan completed the writing and statistical analysis of the present study. Coauthors provided statistics consultation, reviewed, and edited drafts of the manuscript. The National Database for Autism Research (NDAR) data was used for this study.

4.2 Abstract

The transition into adolescence from middle childhood and the process of puberty are normative developmental milestones, yet this time period can be challenging for some youths. Elevated psychopathology symptoms, such as depression, anxiety, conduct problems, and aggression, may become a problem for some adolescents, especially autistic adolescents, who develop heterotypic comorbidity as they transition through pubertal maturation. The present study aimed to examine the links among psychopathology symptoms, repetitive behaviors, and puberty in typically developing and autistic adolescents. Multiple regression analysis was used to examine male-specific outcomes, and then exploratorily tested gender differences among typically developing youth, youth who exhibited some autistic stereotypy, and autistic youths. Autistic adolescents had higher internalizing and externalizing symptoms than typically developing adolescents. Females had higher internalizing symptoms than males. Externalizing symptoms and repetitive behaviors were associated with elevated internalizing symptoms indicating heterotypic comorbidity preponderance for both autistic male and female adolescents. Pubertal status predicted lowered externalizing symptoms for autistic male adolescents and for

youths with stronger verbal ability. Typically developing males who were more developed pubertally had elevated internalizing symptoms. Implications from this study include expanding knowledge on the mechanism of repetitive behaviors as a potential protective factor and pubertal maturation as a risk factor of elevated symptoms severity for both typically developing and autistic adolescents.

---

[1]Iowa State University, Department of Human Development and Family Studies, Ames, Iowa

[2]University of Pittsburgh, Department of Psychiatry, Pittsburgh, Pennsylvania

4.3 Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder in which an individual shows stereotyped, repetitive behaviors and restricted interests and have social communication and reciprocation challenges (Marrus & Constantino, 2020). Even with increasing awareness about ASD, information about pubertal development and comorbidity of other psychopathology disorders is less understood. With recent studies finding elevated internalizing symptoms of depression and anxiety in autistic adolescents, investigating both normative and risk factors are necessary to elucidate pathways leading to declining mental health. This study aimed to examine processes that link puberty, psychopathology symptoms, and restricted and repetitive behaviors in autistic and typically developing adolescents.

A normative biopsychosocial process that all youths undergo is adolescent development, and while adolescence can be an awkward experience for many, some youths have added difficulty during this developmental transition. Biological, physiological, and physical changes associated with puberty are also normative, and for some youth, puberty is a developmental period when psychological and behavioral challenges arise and exacerbate (Picci & Scherf,

2015). Adolescent-onset of depression has been reported in youths ages 12 to 17 years, 13.6% in males and 36.1% in females (Breslau et al., 2017). Anxiety disorders also contribute to rise in internalizing problems during adolescence (Wright, Hostinar, & Trainor, 2019; Ohannessian, Milan, & Vannucci, 2017), and specifically for autistic youths, several studies have found elevated internalizing disorders (Hudson, Hall, & Harkness, 2019; van Steensel & Heeman, 2017; Solomon, Miller, Taylor, Hinshaw, & Carter, 2012; Mazzone et al., 2013). The rate of depression are approximately four times higher for autistic youths than typically developing youths and appears to be associated with IQ levels (Hudson, Hall, & Harkness, 2019). Higher rates of anxiety symptoms were also found in autistic youths (van Steensel & Heeman, 2017). Emerging research are finding evidence of heterotypic comorbidity of externalizing and internalizing behaviors with ASD among adolescents (Mazzone, Ruta, & Reale, 2012; Jamison & Schuttler, 2015; Tureck, Matson, May, Whiting, & Davis, 2014). While internalizing symptoms are generally more preponderant in females than males (Zahn-Waxler et al., 2015; Graber, 2013), a moderate but growing rate of male adolescents, specifically autistic male adolescents, experience declining mental health (Bitsika & Sharpley, 2015; Mayes, Calhoun, Murray, & Zahid, 2011). Certain comorbid behaviors (e.g., ADHD, oppositional defiant disorder, conduct disorder) may mask internalizing problems for typically developing and autistic male adolescents. Studies on between male comparisons are needed to investigate risks for developing elevated internalizing problems in typically developing and autistic male adolescents, and there is a dearth of research literature particularly on puberty.

Puberty is a developmentally sensitive period when neurobiological changes occur in the brain, adrenal cortex, and gonads that result in secondary sexual characteristic development, or for short, ‘physical changes.’ Physical changes vary based on youth’s biological sex, genetic

underpinnings, and environmental influences (Van Hulle et al., 2015; Grotzinger et al., 2018). Puberty is a window of adolescent development, and within this window many changes occur quite rapidly and variability of these changes relative to typical averages is an important consideration.

Generally, developing adolescents reach peak height velocity – growth spurt reaches its peak—at around Tanner stage 4 in males and Tanner stage 3-4 in females (Pinyerd & Zipf, 2005; Berenbaum, Beltz, & Corley, 2015). Male youths begin growing body hair (armpit, pubic) at around Tanner stage 2-3 as well as for female youths (Brix et al., 2019). Female youths' breast development sometimes can begin before menarche, and its development continues throughout Tanner stages (Brix et al., 2019). On average, female adolescents begin menarche at around age 12 years, based on a recent longitudinal U.S. cohort study (Biro et al., 2018), and recent research have shown significant differences across race (Biro et al., 2018; Reagan, Salsberry, Fang, Gardner, & Pajer, 2012) as well as socioeconomic status differences among races (Deardorff, Abrams, Ekwaru, & Rehkopf, 2014). Voice change to adult-like vocals in male youths usually settles at around Tanner stage 3-4 (Harries, Walker, Williams, Hawkins, & Hughes, 1997). Each of these physical developmental changes are associated with the hypothalamus-pituitary-adrenal (HPA) and hypothalamus-pituitary-gonadal (HPG) axes and putatively, these axes' tertiary hormones (e.g., testosterone, estrogen, dehydroepiandrosterone; Romeo, 2010; Bordini & Rosenfield, 2011). However, pubertal development that includes the HPA and HPG axes mapping onto physical changes can vary across youths, and it is important to account for that variance within individuals. In males, for example, voice deepening is linked to gonadal maturation, while acne is linked to adrenal maturation (Shirtcliff et al., 2009), and these changes can vary from one male to another.

Within the window of puberty, elevated internalizing and externalizing (for brevity, psychopathology) symptoms appear in some youths. From previous studies, evidence of elevated psychopathology symptoms is found in some early maturing youths (i.e., advanced pubertal status) and vary by gender (Mendle & Ferrero, 2012; Negriff & Susman, 2011; Ge & Natsuaki, 2009; Graber, 2013). Though one study found no difference in pubertal onset in males and pubertal timing in females between typically developing and autistic youths (May, Pang, O'Connell, & Williams, 2017), theories deriving from various studies point to heterotypic psychopathology problems at around puberty and particularly for youths with neurodevelopmental differences (Green, Flash, & Reiss, 2019). For autistic youths, puberty is a double hit of early neuronal perturbations during prenatal and postnatal development as the first hit (Picci & Scherf, 2015). According to Picci and Scherf's (2015) two-hit model of autism, the second hit during puberty encompasses a variety of risk factors that both typically developing and autistic youths have or experience, and divergence of elevated psychopathology symptoms is exacerbated more so for autistic adolescents as puberty initiates. A further investigation into behavioral changes and arising psychopathology symptoms is warranted to see if current psychopathology models and developmental models apply to autistic youths as well as investigation of sex as a biological variable.

Stereotyped and repetitive behaviors are a common feature of autism and have been found to be elevated with psychiatric symptoms (Stratis & Lecavalier, 2013). Very few studies currently exist examining the associations between repetitive behaviors and psychopathology symptoms that also account for gender and then compare with typically developing youths. In the present study, we take a closer look into this specific feature of autism and examine its association with psychopathology symptoms.

The aim of the present study was to test current psychopathology and adolescent developmental models usually applied in research on typically developing adolescents and test these models with autistic adolescents and within gender (males) to compare. We focused first within male adolescents and then, examined gender effects on psychopathology symptoms. First, we confirmed whether or not autistic male adolescents showed elevated internalizing symptoms (e.g., depression, anxiety). Controlling for verbal and adaptive ability, we hypothesized autistic male adolescents show higher elevated internalizing symptoms than typically developing adolescents; we then examined gender effects. Second, we tested whether male adolescents showed elevated internalizing symptoms when accounting for externalizing symptoms. Next, we examined the association between psychopathology symptom domains (internalizing and externalizing) and repetitive behaviors in typically developing and autistic male adolescents and then examined gender effects in each group. We hypothesized that elevated psychopathology symptoms in both domains are associated with elevated repetitive behaviors in autistic male adolescents. Third, we examined pubertal status and pubertal timing and their association with psychopathology symptoms and repetitive behaviors in typically developing and autistic male adolescents. We hypothesized that mature pubertal status and pubertal timing are positively associated with increasing psychopathology symptoms in both domains and that symptoms exacerbation is more pronounced in autistic male adolescents than typically developing male adolescents. We explored gender differences in pubertal status and pubertal timing and specific psychopathology symptoms in autistic adolescents in comparison to typically developing adolescents.

4.4 Methods

4.4.1 Participants

Data and research tools used in the preparation of this manuscript were obtained from the NIH-supported National Database for Autism Research (NDAR). NDAR is a collaborative informatics system created by the National Institutes of Health to provide a national resource to support and accelerate research in autism. Dataset identifier(s): [NIMH Data Archive Collection ID(s) or NIMH Data Archive Digital Object Identifier (DOI)]. This manuscript reflects the views of the authors and may not reflect the opinions or views of the NIH or of the Submitters submitting original data to NDAR. This multi-site study recruited children and adolescents with Autism Spectrum Disorder (ASD) between the ages 8 to 17 years and typically developing children matched in age and genders. The study also included non-autistic siblings of children with ASD as well as parents. Siblings and parents were not the focus of the present study and excluded.

For the ASD group, inclusion criteria for the study were children and adolescents who met ASD criteria through the Autism Diagnostic Interview – Revised (ADI-R), Autism Diagnostic Observation Schedule – Module 2 (ADOS-2), a clinical diagnosis by a clinician, or met criteria in the past and have an IQ score greater than 70. The Autism Diagnostic Observation Schedule, 2nd Edition – Module 3 (Lord et al., 2012) is a measure of disability symptoms, which is considered the “gold standard” measuring tool for autism spectrum disorder. This is a widely used tool to assess communication, social interaction, play, and imagination in children who receive a diagnosis of ASD (Gotham, Risi, Pickles, & Lord, 2007).

Exclusions were any known genetic condition in addition to ASD, medical conditions, clinically significant visual or auditory impairment, neurological disorders, history of significant

prenatal or birth injury, history of neonatal brain damage, any severe nutritional or psychological deprivation, active seizures within the past year, current use of certain medications (benzodiazepine, barbiturate, or anti-epileptic), twin status, and pregnancy. For the typically developing (TD) group, inclusion criteria included no diagnosis of ASD or sibling with ASD and have IQ score greater than 70. Exclusions were diagnosis, referral, or suspected ASD, schizophrenia, learning or intellectual disability, or other developmental or psychiatric disorder, first- or second-degree relative with ASD, a cutoff score of 60 or greater on the Social Responsiveness Scale (SRS), a raw score greater than 11 on the Social Communication Questionnaire (SCQ) Lifetime, a Child and Adolescent Symptom Inventory (CASI) score in the clinical range for a psychiatric disorder, active seizures within the past year, any current use of medications (same as above), twin status, and pregnancy.

Those in the ASD group were youths who met clinical criteria for an ASD diagnosis (dx) based off of the ADOS (raw scores ≥7 in the social affect + restricted and repetitive behavior domain), and those who did not meet criteria for an ASD dx but were below cutoff [raw score of ≥ 3 and ≤ 6 on the ADOS (Kamp-Becker et al., 2018) or had a T score ≥ 55 on the Social Responsiveness Scale (Frazier et al., 2012)] were included in the data and analysis. Some youths mask their symptoms/behaviors or that these symptoms/behaviors are masked by another comorbid disorder ADHD (Kentrou, de Veld, Mataw, & Begeer, 2019). It is important to note that clinicians use reliable and validated tools to assess youths for ASD, and even so, there are some youths who may not meet criteria for a diagnosis using these assessment tools due to mild symptoms/behaviors (Begeer et al., 2013) or variability in diagnosis by the assessor (Kamp-Becker et al., 2018). Due to certain limitations in health care benefits (e.g., insurance), many people rarely seek a “second opinion” because of the cost burden of evaluations.

Those who met criteria for a diagnosis of ASD were 176 youths, who were mean age 12.14 years (SD = 2.78, range 8.00—17.83, females N = 70, males N = 106). Those who did not meet criteria for an ASD dx but scored just below cutoff on the ADOS and SRS were included in the data (N = 55, 12.2% of the total sample); their mean age was 13.86 (SD = 2.88, range 8.08—18.00, females N = 33, males N = 22). The TD group consisted of 219 youths, who were mean age 13.02 years (SD = 3.01, range 8.00—17.92, females N = 112, males N = 107). Family demographic information (parent education, income, and race) is shown in Table 4.10.1.

4.4.2 Measures

All measures' mean, standard deviation, and total number of valid data by gender and group are reported in Table 4.A in the Supplement section, including measures for control variables verbal ability and living skills adaptive ability. The subscores for communication and for living skills adaptive abilities from the Vineland Adaptive Behavior Scale (VABS; Sparrow, 2011) were used as control variables. Total subscores for communication and living skills adaptive abilities were standardized to capture deviations from the grand mean.

4.4.2.1 Repetitive Behaviors

Repetitive behaviors were identified through parent reports using the Repetitive Behavior Scale – Revised (RBS-R), a common measurement tool to assess individuals on the autism spectrum on repetitive behaviors (Lam & Aman, 2007). The RBS-R has 6 subscales (stereotyped behavior, self-injurious behavior, compulsive behavior, routine behavior, sameness behavior, and restricted behavior). Each subscale was combined into a total score to represent repetitive behaviors.

4.4.2.2 Psychopathology Symptoms

The Child Behavior Checklist (Achenbach & Rescorla, 2001; Dutra, Campbell, & Westen, 2004) was used to assess youth's psychopathology symptoms, and for this study, parents reported for the CBCL. The CBCL includes subscales for activities, social, anxious/depressed, withdrawn/depressed, somatic complaints, rule-breaking behavior, and aggressive behavior that are divided into three domains: social functioning, mood and anxiety symptoms, and externalizing symptoms. We selected internalizing and externalizing symptoms domain. For the internalizing domain, a total score combined the anxious/depressed, withdrawn/depressed, and somatic complaints subscales. For the externalizing domain, rule-breaking and aggressive behaviors subscales were combined into a total score.

In addition to the CBCL, the Child and Adolescent Symptom Inventory (CASI) was included for disorder-specific psychopathology symptoms. Parents reported for the CASI. There are four scores calculated for the CASI: symptom count, symptom severity, impairment, and clinical cutoff. For this study, we selected the symptom count scores as a reflection of symptoms that generally fit criteria for psychiatric problems according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). We included in this study symptoms count for oppositional defiant disorder (ODD) and conduct disorder (CD), separately to represent externalizing disorders; and included generalized anxiety disorder (GAD) and major depressive disorder (MDD), separately to represent internalizing disorders.

4.4.2.3 Pubertal Status

Youths and parents reported pubertal changes using the Pubertal Development Scale (PDS; Petersen et al., 1988). Youth-reported and parent-reported PDS were correlated at $r = 0.842$ ($N = 229$). High concordance between respondents were expected. The PDS was converted

to the Tanner stage metric derived from an SPSS syntax from Shirtcliff and colleagues (2009). This formula converts the PDS to Tanner stages that map onto gonadal and adrenal development during puberty. A pubertal stage/status score that combines gonadal and adrenal development mapping onto Tanner stages was calculated within the PDS informant (youth, parent). Due to a high concordance in the PDS reports between informants, we combined their scores as an average score. TD youths were on average pubertal stage 2.91 (SD = 1.18, 50% within group were stage 3.25); youths who were *below ASD dx cutoff* on average were pubertal stage 3.40 (SD = 1.23, 50% within group were stage 3.75), and autistic youths were on average pubertal stage 2.66 (SD = 1.11, 50% within group were stage 2.63). An ANOVA was performed to test differences among the three groups in pubertal status, which showed significant difference among groups [$F(2, 228) = 4.335$, $p = 0.014$]. Bonferroni post-hoc analysis showed the differences were between adolescents who were *below ASD dx cutoff* and TD adolescents (M difference = -0.74, $p = 0.013$) and between adolescents who were *below ASD dx cutoff* and autistic adolescents (M difference = -0.74, $p = 0.013$). In the overall sample with a stage range of 1-5, male adolescents were mean pubertal stage 2.70 (SD = 1.13), and female adolescents were mean pubertal stage 3.05 (SD = 1.20). Within males, no group differences in pubertal stage was found [$F(2,120) = 0.343$, $p = 0.710$); however, a significant difference among group was found within females [$F(2, 107) = 5.306$, $p = 0.006$). Bonferroni post-hoc test was performed and showed the differences were between TD females and females who were *below ASD dx cutoff* (M difference = -0.86, $p = 0.043$) and between autistic females and females who were *below ASD dx cutoff* (M difference = 1.17, $p = 0.005$).

4.4.3 Analytic Strategy

We conducted multiple linear regression analyses using SPSS 26.0 (IBM Corp., 2019). In testing effects of the overall sample, we ran linear regressions with independent variables entered in the same block and included control variables in a second block using the stepwise method. If control variables did not significantly contribute to the prediction of the dependent variable, the control variables were automatically removed from the final parameters model (Field, 2013). Analyses were performed in three separate methods: 1) Investigation of within-males analyses excluded females in the data by selecting males only in the data. 2) Gender and group comparisons of the full sample were then tested. 3) Lastly, where gender and group comparisons showed significant effects, linear regressions were performed by splitting the data by gender (female, male) and group (ASD, *below ASD dx cutoff*, TD) to investigate where gender and group differences lie.

Durbin-Watson test statistic was used to determine correlations between errors, and any models with values above 3 or below 1 were not included in final results. Results are reported below with unstandardized beta coefficient, standard error, *t*-test, p-value, and number of adolescents. Pairwise deletions of missing data were performed during data analysis; therefore, number of participants included after deletions were reported with the results.

4.5 Results

4.5.1 Do ASD and TD male adolescents show differences in elevated internalizing symptoms and is there a gender difference?

We first tested the hypothesis that autistic male adolescents showed higher internalizing symptoms than TD male adolescents. Next, we tested gender and group (TD, *below ASD dx cutoff*, ASD) effects on internalizing symptoms (CBCL). We controlled for verbal ability and

living skills adaptive ability (VABS) in the regression model by adding these variables in the second block using stepwise method.

In the overall sample, autistic adolescents had more elevated internalizing symptoms compared to typically developing adolescents ($b = 15.258$, $SE = 1.11$, $t = 13.76$, $p < 0.001$, $N = 416$). Verbal ability and living skills adaptive ability did not significantly predict elevated internalizing symptoms ($ps > 0.158$). Within gender (filtered data for males only), autistic adolescents had more elevated internalizing symptoms than TD adolescents ($b = 15.747$, $SE = 1.39$, $t = 11.35$, $p < 0.001$, $N = 211$).

We, then, tested gender and group effects on internalizing symptoms in the overall sample and included in the regression model the variables: gender, group, and gender x group interaction variables. Gender effect on internalizing symptoms was significant ($b = 3.265$, $SE = 1.43$, $t = 2.29$, $p = 0.023$, $N = 412$). Group effect on internalizing symptoms was significant ($b = 15.339$, $SE = 1.49$, $t = 10.31$, $p < 0.001$, $N = 416$). Gender x group interaction effect on internalizing symptoms was non-significant ($p = 0.75$). This finding indicated that autistic female adolescents had more elevated internalizing symptoms than ASD and TD males.

4.5.2 What are the associations among internalizing and externalizing symptoms and repetitive behaviors between genders and adolescent group?

Externalizing symptoms and repetitive behaviors predicted internalizing symptoms in adolescents. We, then, tested the hypothesis that elevated externalizing symptoms and repetitive behaviors predicted increasing internalizing symptoms. In the overall sample, externalizing symptoms predicted more elevated internalizing symptoms ($b = 0.633$, $SE = 0.052$, $t = 12.19$, $p < 0.001$, $N = 416$). Repetitive behaviors predicted more elevated internalizing symptoms ($b = 0.158$, $SE = 0.034$, $t = 4.64$, $p < 0.001$, $N = 371$) as well as more elevated externalizing symptoms ($b = 0.419$, $SE = 0.026$, $t = 15.93$, $p < 0.001$, $N = 371$).

Externalizing symptoms and repetitive behaviors predicted internalizing symptoms in males. We filtered males only in the data, split data by group, and tested a regression with externalizing symptoms and repetitive behaviors predicting internalizing symptoms. Externalizing symptoms predicted more elevated internalizing symptoms for TD and ASD males, and a trend effect was found for *below ASD dx cutoff* males (TD: b = 0.586, SE = 0.12, t = 4.80, p < 0.001, N = 97; *below ASD dx cutoff:* b = 0.514, SE = 0.26, t = 1.98, p = 0.076, N = 19; ASD: b = 0.323, SE = 0.09, t = 3.81, p < 0.001, N = 95). See Figure 4.11.1. Repetitive behaviors predicted more elevated internalizing symptoms for TD males, and a trend effect was found for ASD males (TD: b = 0.632, SE = 0.25, t = 2.56, p = 0.012, N = 88; *below ASD dx cutoff:* b = 0.238, SE = 0.13, t = 1.78, p = 0.105, N = 16; ASD: b = 0.095, SE = 0.05, t = 1.96, p = 0.053, N = 90).

Externalizing symptoms and repetitive behaviors predicted internalizing symptoms in males and females. We then examined gender and group differences in externalizing symptoms and repetitive behaviors effect on internalizing symptoms. A trend effect of gender on externalizing symptoms was found (b = 1.694, SE = 0.88, t = 1.93, p = 0.054, N = 412), and a significant group effect on externalizing symptoms was found (b = 6.625, SE = 1.19, t = 5.56, p < 0.001, N = 416). Externalizing symptoms as well as repetitive behaviors were found to significantly predict internalizing symptoms (b = 0.56, SE = 0.05, t = 10.86, p < 0.001, N = 416; b = 0.085, SE = 0.04, t = 2.36, p = 0.019, N = 371, respectively). We, then, split data by gender and group to further examine differences in effects. Results are displayed in Table 4.10.2. The effect of externalizing symptoms on internalizing symptoms were found for TD males and females as well as ASD males and females, but only a trend effect was found for males and females *below ASD dx cutoff.* Based on the effect sizes (beta coefficient), autistic females had the

most elevated internalizing symptoms closely followed by males *below ASD dx cutoff.* TD males and males *below ASD dx cutoff* had more elevated internalizing symptoms than ASD males. Repetitive behaviors showed strongest effect on internalizing symptoms for TD males and females than males and females in the ASD group and *below ASD dx cutoff group.* Increasing repetitive behaviors for autistic adolescents did not impact internalizing symptoms, but rather was found for TD adolescents.

Repetitive behaviors predicted externalizing symptoms in males and in females. We, then, tested repetitive behavior as a predictor of externalizing symptoms within male adolescents and split data by group. Repetitive behaviors in all three male groups predicted elevated externalizing symptoms (TD: $b = 0.611$, $SE = 0.21$, $t = 2.94$, $p = 0.004$, $N = 88$; *below ASD dx cutoff*: $b = 0.285$, $SE = 0.13$, $t = 2.22$, $p = 0.049$, $N = 16$; ASD: $b = 0.293$, $SE = 0.05$, $t = 5.64$, $p < 0.001$, $N = 90$). Next, we tested whether gender, group, and the interaction between gender x group had an effect on externalizing symptoms while controlling for repetitive behaviors. Gender effect was found on externalizing symptoms ($b = 2.627$, $SE = 1.23$, $t = 2.14$, $p = 0.033$, $N = 450$) as well as a group effect on externalizing symptoms was found to be significant ($b = 5.359$, $SE = 1.46$, $t = 3.66$, $p < 0.001$, $N = 416$). Gender x group interaction effect was non-significant ($p = 0.885$). These results indicated that females had more elevated externalizing symptoms than males and that ASD adolescents showed more elevated externalizing symptoms than TD adolescents, while controlling for repetitive behaviors. The effect of repetitive behaviors on externalizing behaviors persisted with covariates gender and group in the regression model ($b = 0.339$, $SE = 0.03$, $t = 10.67$, $p < 0.001$, $N = 371$). A visual representation of the results is in Figure 4.11.2.

4.5.3 Does pubertal status affect elevated psychopathology symptoms and repetitive behaviors and is there a gender and group difference?

We tested the hypothesis that pubertal status is associated with elevated psychopathology symptoms and repetitive behaviors in TD and autistic adolescents and explored gender differences in this hypothesis model. Puberty scores were calculated as stage (see Method section). These stages represent pubertal status. A variable pubertal timing was created by residualizing chronological age on pubertal status and then saving the residuals (Mendle et al., 2019). Pubertal stage/status effect would indicate the pubertal stage at which the adolescent show elevated symptoms. Pubertal timing effect would be an indicator that the adolescent who is more advanced in pubertal development relative to peers of the same age. We examined both pubertal status and pubertal timing in all subsequent regressions.

In the overall sample, pubertal status did not significantly predict elevated internalizing symptoms ($p = 0.762$, $N = 223$), externalizing symptoms ($p = 0.370$, $N = 223$), and repetitive behaviors ($p = 0.265$, $N = 200$). Pubertal timing also did not significantly predict internalizing symptoms ($p = 0.599$, $N = 190$), externalizing symptoms ($p = 0.537$, $N = 190$), and repetitive behaviors ($p = 0.776$, $N = 171$).

Within male adolescents only and split data by group, pubertal status had an effect on elevated internalizing symptoms only for TD males ($b = 2.313$, $SE = 1.06$, $t = 2.18$, $p = 0.034$, $N = 54$), but not for ASD males and males *below ASD dx cutoff* ($ps > 0.544$). Pubertal timing did not show any effect in all three groups ($ps > 0.369$). Pubertal status had a significant effect on externalizing symptoms for autistic adolescents ($b = -3.537$, $SE = 1.35$, $t = -2.61$, $p = 0.012$, $N = 52$). The control variable verbal ability also showed significant effect on externalizing symptoms in this model ($b = -4.249$, $SE = 1.67$, $t = -2.54$, $p = 0.014$, $N = 92$). Autistic male adolescent who had mature pubertal status showed decreasing externalizing symptoms as well as those who had

better verbal ability. Pubertal timing did not significantly predict externalizing symptoms (ps > 0.415), nor repetitive behaviors (ps > 0.133) in all three groups. Pubertal status did not significantly predict repetitive behaviors (ps > 0.567).

We, then, took a deeper dive into these findings and examined whether puberty predicted symptoms/behaviors by gender and group comparisons by splitting the data. Pubertal status had a significant effect on internalizing symptoms only for TD males (b = 2.313, SE = 1.06, t = 2.18, p = 0.034, N = 54) but not for ASD and *below ASD dx cutoff* males and females (ps > 0.544). Pubertal status had a significant effect on externalizing symptoms for females *below ASD dx cutoff* and for ASD males (b = -5.501, SE = 2.18, t = -2.52, p = 0.028, N = 13; b = -3.537, SE = 1.35, t = -2.61, p = 0.012, N = 52, respectively). Verbal ability effect also was significant in this regression model (b = -4.249, SE = 1.67, t = -2.54, p = 0.014, N = 92). In this model, the findings showed not only autistic males with more mature pubertal status had decreasing externalizing symptoms, females *below ASD dx cutoff* showed the same pattern.

Lastly, we examined gender and group comparisons of puberty predicting selected psychopathology symptoms by category (ODD, CD, GAD, and MDD) using the CASI symptoms count. Autistic adolescents showed elevated ODD symptoms compared to TD adolescents (b = 2.111, SE = 0.294, t = 7.18, p < 0.001, N = 378). Gender and pubertal status did not predict elevated ODD symptoms (ps > 0.301). Autistic adolescents showed elevated CD symptoms compared to TD adolescents (b = 0.511, SE = 0.11, t = 4.83, p < 0.001, N = 378). Gender and pubertal status did not predict elevated ODD symptoms (ps > 0.441). Autistic adolescents had more elevated generalized anxiety symptoms than TD adolescents (b = 0.772, SE = 0.09, t = 8.36, p < 0.001, N = 373). A significant effect of gender on generalized anxiety symptoms (b = 0.187, SE = 0.09, t = 2.17, p = 0.031, N = 372) indicating female adolescents

showed more elevated generalized anxiety symptoms than male adolescents in the overall sample. Pubertal status did not predict GAD ($p = 0.248$). Autistic adolescents showed higher elevated depressive symptoms than TD adolescents ($b = 0.610$, $SE = 0.115$, $t = 5.28$, $p < 0.001$, $N = 378$). Gender and pubertal status did not predict elevated depressive symptoms ($ps > 0.121$).

4.6 Discussion

A comparison between gender and group of typically developing and autistic youths was investigated to examine differences in internalizing, externalizing, and repetitive behaviors. Pubertal status and pubertal timing were tested as moderators of symptoms severity. We explored gender differences as well as included a group of youths who did not meet clinical cutoff for an ASD diagnosis and presented with mild ASD behaviors that during an evaluation, ASD-related behaviors may have missed. We controlled for verbal and living skills adaptive ability in all regression models. This study utilized a public and accessible dataset from the National Institute on Mental Health Data Archive (https://nda.nih.gov/) that included a range of psychopathology and autism measures that allowed for investigating multiple psychopathology outcomes.

The autism group overall had higher internalizing, externalizing, and repetitive behaviors than typically developing youths, which was previously found in other studies (Hudson, Hall, & Harkness, 2019; van Steensel & Heeman, 2017). Autistic adolescents altogether had higher specific psychopathology symptoms (oppositional defiance, conduct disorder problems, generalized anxiety, depression) than typically developing adolescents. The present study adds to a growing research literature that autistic adolescents are vulnerable to comorbidity of psychiatric disorders (Mazefsky et al., 2012; Moseley, Tonge, Brereton, & Einfeld, 2011; Rosen, Mazefsky, Vasa, & Lerner, 2018).

Acknowledgement of heterotypic comorbidity during adolescent development and in males is important when studying increases in internalizing symptoms. High comorbidity of externalizing and internalizing symptoms in male adolescents has been confirmed in several studies (Lahey et al., 2018; Carragher et al., 2016) as well as for autistic adolescents (Patalay et al., 2015; Noordhof et al., 2015; Mazefsky et al., 2012). Externalizing symptoms was positively associated with internalizing symptoms for TD and ASD youths. Autistic females who had high externalizing symptoms had the highest elevated internalizing symptoms in the overall sample. Autistic males who had high externalizing symptoms had a significant increase in internalizing symptoms, but the effect was not as strong as for autistic females. Typically developing males and females who had high externalizing symptoms also had increasing internalizing symptoms. These findings indicate group status had less of an effect than the comorbid interrelations between externalizing and internalizing symptoms. As found in this study, heterotypic comorbidity severity affects all youths regardless of neurodevelopmental and gender differences. Generally, externalizing symptoms are preponderant in male adolescents; however, as found in this study, female adolescents also had high externalizing symptoms and was found to be comorbid with internalizing symptoms. This heterotypic comorbidity was ascribed to autistic females rather than typically developing females.

A relatively under-investigated behavior in association with internalizing and externalizing symptoms is repetitive behaviors, sometimes referred to as “stimming.” Autistic individuals all participate in some form of repetitive behavior and even typically developing individuals engage as well but usually not to the same degree as autistic individuals. In this study as baseline, autistic youths overall engaged in more repetitive behavior than typically developing youths. Repetitive behavior only moderately predicted elevated internalizing symptoms in

autistic males, while for typically developing males, repetitive behavior was associated with elevated internalizing symptoms. The association between repetitive behavior and internalizing symptoms may be more correlative than causative; future research is needed to distinguish the nature of this association. A potential explanation for repetitive behavior showing a moderate effect on internalizing symptoms for autistic males is that autistic individuals are habituated to repetitive behaviors. Increases in internalizing symptoms may be better explained by other stress-related factors for autistic adolescents. Since repetitive behavior is less common in typically developing individuals, it may coincide with increasing internalizing symptoms as a form of coping. There may be benefits of repetitive behaviors as a coping strategy when elevated in stressful conditions or environments, as was reported in a phenomenological study (Manor-Binyamini & Schreiber-Divon, 2019). A strong association between elevated restricted and repetitive behaviors and elevated psychiatric comorbidities was previously found (Stratis & Lecavalier, 2013; Antezana et al., 2019), which could also suggest that repetitive behavior's association with elevated psychopathology symptoms could also be maladaptive. There is currently a dearth of research literature on this topic. More research is needed to investigate the mechanism of restricted and repetitive behaviors in autistic and in typically developing adolescents as a mechanism for coping and managing internalizing symptoms.

To date, the investigation of repetitive behavior and externalizing symptoms effects on internalizing symptoms is relatively understudied. In the present study, we found that both repetitive behaviors and externalizing symptoms together predicted elevated internalizing symptoms in the overall sample. For males, autistic adolescents had a high baseline of internalizing symptoms relative to TD adolescents (intercept: $b = 40.08$ vs $b = 19.06$ in symptom severity score). Regardless of internalizing symptoms severity starting point, TD males with high

externalizing symptoms, controlling for repetitive behavior, had a stronger increase in internalizing symptoms than for autistic males and males *below ASD dx cutoff*. The findings from this study suggest that heterotypic comorbidity for TD males may be more maladaptive than it is for autistic males, but this is not to say that the finding for autistic males mean they are more resilient. As mentioned above, autistic individuals who engage in repetitive behaviors regularly may be habituated to its function, and the same description could be applied to autistic males with high externalizing symptoms. Features of externalizing symptoms, such as conduct problems, oppositional defiance, and overt aggression, may also be forms of masking internalizing symptoms for autistic males. To better understand the mechanism of heterotypic comorbidity and autism, research is needed to disentangle each of these features and consider sex as a biological variable since presentation of symptoms may vary by biological sex.

In the present study, we found that typically developing adolescent males with more mature pubertal status had elevated internalizing symptoms but not for autistic adolescents. However, we found that autistic males and females *below ASD dx cutoff* who were more developed pubertally had decreasing externalizing symptoms while controlling for verbal ability. Stronger verbal ability and more mature pubertal status appeared to be protective for autistic males from developing internalizing symptoms and especially in lessening externalizing problems. On the topic of verbal ability, a different study had contrary findings that found greater verbal ability association with elevated internalizing and externalizing symptoms in a clinically-referred sample of autistic youths, predominantly male (Lerner et al., 2018). Contrary findings between the present study and Lerner and colleagues' (2018) study could be a difference in participant sample (i.e., clinical vs. community) as well as differences in measurements of verbal ability and psychopathology symptoms. It may be important to note that autistic males in

this sample overall were younger in age as well as less developed pubertally than typically developing males. Even so, autistic males did not show elevated psychopathology symptoms due to more advanced puberty; rather, advanced puberty for autistic male adolescents had either no effect on psychopathology symptoms or a lessening of symptoms. Elevated psychopathology symptoms in autistic adolescents in this sample appeared to be ascribed to other unmeasured factors in this study. Future studies are recommended to investigate adolescent-related stressors' effects on heterotypic comorbidities in autistic adolescents, such as social and ecological systemic effects, peer-to-peer interactions, platonic and romantic relationships, identity affiliation, and family environment.

As emerging research on heterotypic comorbidity and autistic behaviors increase, several sampling procedures are worth mentioning. Studies on this topic are rare, and if any exist, results on gender and symptoms severity appear to be mixed. Few studies found gender differences in psychopathology comorbidities in adolescents. For example, one study on a small number of young adult group of autistic individuals found a prevalence of mood disorders higher in females than in males (Kreiser & White, 2015). Conversely, a different study, that is comparative in sample size but a study on children and adolescents, found male youths had higher comorbid anxiety symptoms than female youths (Mannion & Leader, 2013). However, two other studies with larger sample sizes, including a large community sample of over 4,000 children and adolescents, did not find a gender difference in comorbidity of psychiatric disorders in autistic youths (Worley & Matson, 2011; Rosenberg, Kaufmann, Law, & Law, 2011). More current research is needed on a community-based sample of adolescents and an investigation of comorbid psychopathology symptoms to track trends of stability or change. Also, it is important

for future research on clinical samples of autistic adolescents to consider sex as a biological variable.

4.7 Implications for Targeted Interventions

A current gap in research translation to preventative interventions with the autism population is targeting both maladaptive autistic behaviors alongside comorbidity of psychiatric conditions. Treatment interventions for autistic youths mostly target autistic behaviors or specific psychopathologies with limited expertise in comorbidity of autism and psychiatric disorders (Hepburn, Stern, Blakeley-Smith, Kimel, & Reaven, 2014). Unintended consequences, such as low efficacy of psychiatric treatments (Nahar, Thippeswamy, Shanker Reddy, Kishore, & Chaturvedi, 2019), can be anticipated when treatments are not targeted toward comorbid psychiatric conditions with ASD. A precursory issue and gap between research and clinical practice is that limited knowledge on understanding pathways linking to risk factors of psychiatric comorbidities in adolescents. In building efficacious interventions, a better understanding of mechanisms leading to symptoms comorbidity in autism is needed, specifically in adolescence.

4.8 Limitations

Several strengths of the present study should be noted. This was the first study to examine pubertal status and timing effects on repetitive behaviors and internalizing and externalizing symptoms in adolescents. We took a unique approach in investigating symptoms and behavior change by first examining within gender (males) and then tested gender differences. The inclusion of adolescents who did not meet criteria for an ASD diagnosis but close to the clinical cutoff was also a unique perspective at investigating symptoms severity, especially because there are likely a substantial number of youths who may not meet criteria for

an ASD diagnosis but could be largely understudied that may need more research attention. The present study has shown that this particular subgroup of youths had elevated symptoms severity.

Some limitations of this study are worth noting. Other variables that could be risk factors of symptoms severity were not tested and beyond the scope of the present study, and if tested, may be important to investigate using a within-gender and then gender difference approach at examining symptoms type and severity. A second limitation of the study is due to the data being cross-sectional, it limits an understanding of symptoms type and severity change or stability as youths mature and age. A recommendation for future research is to use this study's approach in examining within gender and then gender differences between typically developing and autistic adolescents to study symptoms type and severity over time. Lastly, in order to break down the mechanisms of pubertal development and its influence on autistic adolescents who show increasing psychopathology symptoms, stronger measures of puberty are needed. The present study only used the Pubertal Development Scale as a measure of puberty. Tanner staging and pubertal hormones can contribute to a larger understanding of the physical and biological mechanisms of pubertal effects on psychopathology symptoms in the autism population.

4.9 Conclusions

Studies examining internalizing and externalizing symptoms as well as stereotyped and repetitive behaviors in autistic adolescents are still too uncommon and is needed. The present study investigated psychopathology symptoms and autistic behaviors in adolescents first by focusing on differences between typically developing and autistic males and then testing gender differences between groups. Autistic adolescents were higher in internalizing, externalizing, and repetitive behaviors and that repetitive behaviors were associated with increasing internalizing and externalizing symptoms in males. More mature pubertal status was associated with

decreasing externalizing symptoms in autistic male adolescents when controlling for verbal ability. This study's findings contribute to a small literature on puberty effects on autism and psychopathology symptoms severity.

References

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the Achenbach system of empirically based assessment school-age forms profiles.* Retrieved from http://aseba.org/wp-content/uploads/2019/01/ASEBA-Reliability-and-Validity-School-Age.pdf

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders*. https://doi.org/10.1176/appi.books.9780890425596

Antezana, L., Factor, R. S., Condy, E. E., Strege, M. V., Scarpa, A., & Richey, J. A. (2019). Gender differences in restricted and repetitive behaviors and interests in youth with autism. *Autism Research*. https://doi.org/10.1002/aur.2049

Begeer, S., Mandell, D., Wijnker-Holmes, B., Venderbosch, S., Rem, D., Stekelenburg, F., & Koot, H. M. (2013). Sex differences in the timing of identification among children and adults with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-012-1656-z

Berenbaum, S. A., Beltz, A. M., & Corley, R. (2015). The Importance of Puberty for Adolescent Development: Conceptualization and Measurement. In *Advances in Child Development and Behavior*. https://doi.org/10.1016/bs.acdb.2014.11.002

Biro, F. M., Pajak, A., Wolff, M. S., Pinney, S. M., Windham, G. C., Galvez, M. P., … Teitelbaum, S. L. (2018). Age of Menarche in a Longitudinal US Cohort. *Journal of Pediatric and Adolescent Gynecology*, *31*(4), 339–345. https://doi.org/10.1016/j.jpag.2018.05.002

Bitsika, V., & Sharpley, C. F. (2015). Differences in the Prevalence, Severity and Symptom Profiles of Depression in Boys and Adolescents with an Autism Spectrum Disorder versus Normally Developing Controls. *International Journal of Disability, Development and Education*. https://doi.org/10.1080/1034912X.2014.998179

Bordini, B., & Rosenfield, R. L. (2011). Normal pubertal development: part II: clinical aspects of puberty. *Pediatrics in Review*, *32*(7), 281–292. https://doi.org/10.1542/pir.32-7-281

Breslau, J., Gilman, S. E., Stein, B. D., Ruder, T., Gmelin, T., & Miller, E. (2017). Sex differences in recent first-onset depression in an epidemiological sample of adolescents. *Translational Psychiatry*. https://doi.org/10.1038/tp.2017.105

Brix, N., Ernst, A., Lauridsen, L. L. B., Parner, E., Støvring, H., Olsen, J., … Ramlau-Hansen, C. H. (2019). Timing of puberty in boys and girls: A population-based study. *Paediatric and Perinatal Epidemiology*. https://doi.org/10.1111/ppe.12507

Carragher, N., Teesson, M., Sunderland, M., Newton, N. C., Krueger, R. F., Conrod, P. J., … Slade, T. (2016). The structure of adolescent psychopathology: A symptom-level analysis. *Psychological Medicine*. https://doi.org/10.1017/S0033291715002470

Deardorff, J., Abrams, B., Ekwaru, J. P., & Rehkopf, D. H. (2014). Socioeconomic status and age at menarche: An examination of multiple indicators in an ethnically diverse cohort. *Annals of Epidemiology*. https://doi.org/10.1016/j.annepidem.2014.07.002

Dutra, L., Campbell, L., & Westen, D. (2004). Quantifying Clinical Judgment in the Assessment of Adolescent Psychopathology: Reliability, Validity, and Factor Structure of the Child Behavior Checklist for Clinician Report. *Journal of Clinical Psychology*. https://doi.org/10.1002/jclp.10234

Field, A. (2013). Discovering statistics using IBM SPSS statistics. In *Statistics*.

Frazier, T. W., Youngstrom, E. A., Speer, L., Embacher, R., Law, P., Constantino, J., … Eng, C. (2012). Validation of proposed DSM-5 criteria for autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*. https://doi.org/10.1016/j.jaac.2011.09.021

Ge, X., & Natsuaki, M. N. (2009). In search of explanations for early pubertal timing effects on developmental psychopathology. *Current Directions in Psychological Science*. https://doi.org/10.1111/j.1467-8721.2009.01661.x

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The Autism Diagnostic Observation Schedule: Revised Algorithms for Improved Diagnostic Validity. *Journal of Autism and Developmental Disorders*, *37*(4), 613–627. https://doi.org/10.1007/s10803-006-0280-1

Graber, J. A. (2013). Pubertal timing and the development of psychopathology in adolescence and beyond. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2013.04.003

Green, T., Flash, S., & Reiss, A. L. (2019). Sex differences in psychiatric disorders: what we can learn from sex chromosome aneuploidies. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-018-0153-2

Grotzinger, A. D., Briley, D. A., Engelhardt, L. E., Mann, F. D., Patterson, M. W., Tackett, J. L., … Harden, K. P. (2018). Genetic and environmental influences on pubertal hormones in human hair across development. *Psychoneuroendocrinology*, *90*, 76–84. https://doi.org/10.1016/j.psyneuen.2018.02.005

Harries, M. L. L., Walker, J. M., Williams, D. M., Hawkins, S., & Hughes, I. A. (1997). Changes in the male voice at puberty. *Archives of Disease in Childhood*. https://doi.org/10.1136/adc.77.5.445

He, P., Guo, C., Wang, Z., Chen, G., Li, N., & Zheng, X. (2018). Socioeconomic status and childhood autism: A population-based study in China. *Psychiatry Research*. https://doi.org/10.1016/j.psychres.2017.08.046

Hepburn, S. L., Stern, J. A., Blakeley-Smith, A., Kimel, L. K., & Reaven, J. A. (2014). Complex Psychiatric Comorbidity of Treatment-Seeking Youth With Autism Spectrum Disorder and Anxiety Symptoms. *Journal of Mental Health Research in Intellectual Disabilities*. https://doi.org/10.1080/19315864.2014.932476

Hudson, C. C., Hall, L., & Harkness, K. L. (2019). Prevalence of Depressive Disorders in Individuals with Autism Spectrum Disorder: a Meta-Analysis. *Journal of Abnormal Child Psychology*. https://doi.org/10.1007/s10802-018-0402-1

Jamison, T. R., & Schuttler, J. O. (2015). Examining social competence, self-perception, quality of life, and internalizing and externalizing symptoms in adolescent females with and without autism spectrum disorder: A quantitative design including between-groups and correlational analyses Understa. *Molecular Autism*. https://doi.org/10.1186/s13229-015-0044-x

Kamp-Becker, I., Albertowski, K., Becker, J., Ghahreman, M., Langmann, A., Mingebach, T., … Stroth, S. (2018). Diagnostic accuracy of the ADOS and ADOS-2 in clinical practice. *European Child and Adolescent Psychiatry*. https://doi.org/10.1007/s00787-018-1143-y

Kentrou, V., de Veld, D. M. J., Mataw, K. J. K., & Begeer, S. (2019). Delayed autism spectrum disorder recognition in children and adolescents previously diagnosed with attention-deficit/hyperactivity disorder. *Autism*. https://doi.org/10.1177/1362361318785171

Kreiser, N. L., & White, S. W. (2015). ASD Traits and Co-occurring Psychopathology: The Moderating Role of Gender. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-015-2580-9

Lahey, B. B., Zald, D. H., Perkins, S. F., Villalta-Gil, V., Werts, K. B., Van Hulle, C. A., … Waldman, I. D. (2018). Measuring the hierarchical general factor model of psychopathology in young adults. *International Journal of Methods in Psychiatric Research*. https://doi.org/10.1002/mpr.1593

Lam, K. S. L., & Aman, M. G. (2007). The repetitive behavior scale-revised: Independent validation in individuals with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-006-0213-z

Lerner, M. D., Mazefsky, C. A., Weber, R. J., Transue, E., Siegel, M., Gadow, K. D., … Williams, D. (2018). Verbal Ability and Psychiatric Symptoms in Clinically Referred Inpatient and Outpatient Youth with ASD. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-017-3344-5

Lord, C., Rutter, M., DiLavore, P., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism Diagnostic Observation Schedule* (2nd ed.). Retrieved from https://www.wpspublish.com/store/p/2648/ados-2-autism-diagnostic-observation-schedule-second-edition

Mannion, A., & Leader, G. (2013). An analysis of the predictors of comorbid psychopathology, gastrointestinal symptoms and epilepsy in children and adolescents with autism spectrum disorder. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2013.10.002

Manor-Binyamini, I., & Schreiber-Divon, M. (2019). Repetitive behaviors: Listening to the voice of people with high-functioning autism spectrum disorder. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2019.04.001

Marrus, N., & Constantino, J. N. (2016). Autism spectrum disorders. In *The Curated Reference Collection in Neuroscience and Biobehavioral Psychology*. https://doi.org/10.1016/B978-0-12-809324-5.23581-5

Mathew, N. E., Burton, K. L. O., Schierbeek, A., Črnčec, R., Walter, A., & Eapen, V. (2019). Parenting preschoolers with autism: Socioeconomic influences on wellbeing and sense of competence. *World Journal of Psychiatry*. https://doi.org/10.5498/wjp.v9.i2.30

May, T., Pang, K. C., O'Connell, M. A., & Williams, K. (2017). Typical Pubertal Timing in an Australian Population of Girls and Boys with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-017-3281-3

Mayes, S. D., Calhoun, S. L., Murray, M. J., & Zahid, J. (2011). Variables Associated with Anxiety and Depression in Children with Autism. *Journal of Developmental and Physical Disabilities*. https://doi.org/10.1007/s10882-011-9231-7

Mazefsky, C. A., Oswald, D. P., Day, T. N., Eack, S. M., Minshew, N. J., & Lainhart, J. E. (2012). ASD, a Psychiatric Disorder, or Both? Psychiatric Diagnoses in Adolescents with High-Functioning ASD. *Journal of Clinical Child and Adolescent Psychology*. https://doi.org/10.1080/15374416.2012.686102

Mazzone, L., Postorino, V., De Peppo, L., Fatta, L., Lucarelli, V., Reale, L., … Vicari, S. (2013). Mood symptoms in children and adolescents with autism spectrum disorders. *Research in Developmental Disabilities*. https://doi.org/10.1016/j.ridd.2013.07.034

Mazzone, L., Ruta, L., & Reale, L. (2012). Psychiatric comorbidities in asperger syndrome and high functioning autism: diagnostic challenges. *Annals of General Psychiatry*. https://doi.org/10.1186/1744-859X-11-16

Mendle, J., & Ferrero, J. (2012). Detrimental psychological outcomes associated with pubertal timing in adolescent boys. *Developmental Review*, *32*(1), 49–66. https://doi.org/10.1016/J.DR.2011.11.001

Moseley, D. S., Tonge, B. J., Brereton, A. V., & Einfeld, S. L. (2011). Psychiatric Comorbidity in Adolescents and Young Adults With Autism. *Journal of Mental Health Research in Intellectual Disabilities*. https://doi.org/10.1080/19315864.2011.595535

Nahar, A., Thippeswamy, H., Shanker Reddy, M. S., Kishore, M. T., & Chaturvedi, S. K. (2019). Psychiatric comorbidity in persons with high-functioning autism spectrum disorders: Findings from a tertiary care neuropsychiatric hospital. *Asian Journal of Psychiatry*. https://doi.org/10.1016/j.ajp.2018.09.008

Negriff, S., & Susman, E. J. (2011). Pubertal timing, depression, and externalizing problems: A framework, review, and examination of gender differences. *Journal of Research on Adolescence*. https://doi.org/10.1111/j.1532-7795.2010.00708.x

Noordhof, A., Krueger, R. F., Ormel, J., Oldehinkel, A. J., & Hartman, C. A. (2015). Integrating Autism-Related Symptoms into the Dimensional Internalizing and Externalizing Model of Psychopathology. The TRAILS Study. *Journal of Abnormal Child Psychology*. https://doi.org/10.1007/s10802-014-9923-4

Ohannessian, C. M. C., Milan, S., & Vannucci, A. (2017). Gender Differences in Anxiety Trajectories from Middle to Late Adolescence. *Journal of Youth and Adolescence*. https://doi.org/10.1007/s10964-016-0619-7

Patalay, P., Fonagy, P., Deighton, J., Belsky, J., Vostanis, P., & Wolpert, M. (2015). A general psychopathology factor in early adolescence. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.bp.114.149591

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2), 117–133. https://doi.org/10.1007/BF01537962

Picci, G., & Scherf, K. S. (2015). A Two-Hit Model of Autism: Adolescence as the Second Hit. *Clinical Psychological Science, 3*(3), 349–371. https://doi.org/10.1177/2167702614540646

Pinyerd, B., & Zipf, W. B. (2005). Puberty-timing is everything! *Journal of Pediatric Nursing*. https://doi.org/10.1016/j.pedn.2004.12.011

Poulton, R., Caspi, A., Milne, B. J., Thomson, W. M., Taylor, A., Sears, M. R., & Moffitt, T. E. (2002). Association between children's experience of socioeconomic disadvantage and adult health: A life-course study. *Lancet*. https://doi.org/10.1016/S0140-6736(02)11602-3

Reagan, P. B., Salsberry, P. J., Fang, M. Z., Gardner, W. P., & Pajer, K. (2012). African-American/white differences in the age of menarche: Accounting for the difference. *Social Science and Medicine*. https://doi.org/10.1016/j.socscimed.2012.05.018

Romeo, R. D. (2010). Pubertal maturation and programming of hypothalamic-pituitary-adrenal reactivity. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2010.02.004

Rosen, T. E., Mazefsky, C. A., Vasa, R. A., & Lerner, M. D. (2018). Co-occurring psychiatric conditions in autism spectrum disorder. *International Review of Psychiatry*. https://doi.org/10.1080/09540261.2018.1450229

Rosenberg, R. E., Kaufmann, W. E., Law, J. K., & Law, P. A. (2011). Parent Report of Community Psychiatric Comorbid Diagnoses in Autism Spectrum Disorders. *Autism Research and Treatment*. https://doi.org/10.1155/2011/405849

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Solomon, M., Miller, M., Taylor, S. L., Hinshaw, S. P., & Carter, C. S. (2012). Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-011-1215-z

Sparrow, S. S. (2011). Vineland Adaptive Behavior Scales. In *Encyclopedia of Clinical Neuropsychology*. https://doi.org/10.1007/978-0-387-79948-3_1602

Stratis, E. A., & Lecavalier, L. (2013). Restricted and repetitive behaviors and psychiatric symptoms in youth with autism spectrum disorders. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2013.02.017

Tureck, K., Matson, J. L., May, A., Whiting, S. E., & Davis, T. E. (2014). Comorbid Symptoms in Children with Anxiety Disorders Compared to Children with Autism Spectrum Disorders. *Journal of Developmental and Physical Disabilities*. https://doi.org/10.1007/s10882-013-9342-4

Van Hulle, C. A., Moore, M. N., Shirtcliff, E. A., Lemery-Chalfant, K., & Goldsmith, H. H. (2015). Genetic and Environmental Contributions to Covariation Between DHEA and Testosterone in Adolescent Twins. *Behavior Genetics*, *45*(3), 324–340. https://doi.org/10.1007/s10519-015-9709-7

van Steensel, F. J. A., & Heeman, E. J. (2017). Anxiety Levels in Children with Autism Spectrum Disorder: A Meta-Analysis. *Journal of Child and Family Studies*. https://doi.org/10.1007/s10826-017-0687-7

Worley, J. A., & Matson, J. L. (2011). Psychiatric symptoms in children diagnosed with an Autism Spectrum Disorder: An examination of gender differences. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2010.12.002

Wright, E. C., Hostinar, C. E., & Trainor, B. C. (2019). Anxious to see you: Neuroendocrine mechanisms of social vigilance and anxiety during adolescence. *European Journal of Neuroscience*. https://doi.org/10.1111/ejn.14628

Zahn-Waxler, Carolyn, Crick, N. R., Shirtcliff, E. A., & Woods, K. E. (2015). The Origins and Development of Psychopathology in Females and Males. In *Developmental Psychopathology* (pp. 76–138). https://doi.org/10.1002/9780470939383.ch4

4.10 Tables

Table 4.10.1 Demographics on Education, Income, and Youth Race

		Mother	Father
Education		%	%
	Less than high school	2.2	4.2
	High school degree	6.7	11.0
	GED	0.0	2.0
	Some college	20.6	16.2
	Associate degree	6.7	5.4
	Bachelor's degree	28.2	30.6
	Some graduate work	7.7	5.6
	Graduate degree	28.2	25.0
Income		%	%
	$10,000 or less	19.8	5.2
	$10,001 to $15,000	4.2	1.0
	$15,001 to $20,000	4.2	0.5
	$20,001 to $25,000	7.7	4.2
	$25,001 to $30,000	11.6	5.8
	$30,001 to $35,000	14.1	12.0
	$35,001 to $40,000	16.1	18.3
	$40,001 to $45,000	8.9	17.5
	$45,001 to $50,000	9.2	16.5
	$50,001 to $60,000	4.2	18.8

	ASD	Control
Child Race	%	%
American Indian/Alaska Native	0.4	0.0
Asian	2.6	6.4
Black or African American	3.9	8.1
Hawaiian or Pacific Islander	0.4	0.0
More than one race	14.9	9.3
Unknown or not reported	1.3	1.7
Hispanic or Latino	11.0	16.3
White	61.0	57.0

Table 4.10.2 Externalizing Symptoms and Repetitive Behaviors Predicted Internalizing Symptoms: A Group and Gender Comparison

intercept and slopes	Intercept					Externalizing Symptoms					Repetitive Behaviors				
group	b	SE	t	p	N	b	SE	t	p	N	b	SE	t	p	N
TD															
males	19.06	4.96	3.85	<0.001	97	0.59	0.12	4.80	<0.001	97	0.63	0.25	2.56	0.012	88
females	21.50	4.38	4.91	<0.001	107	0.57	0.10	5.70	<0.001	107	0.62	0.26	2.41	0.018	93
below ASD dx cutoff															
males	27.82	13.35	2.08	0.064	19	0.51	0.26	1.98	0.076	19	0.24	0.13	1.78	0.105	16
females	24.19	15.12	1.60	0.125	26	0.64	0.31	2.05	0.054	26	0.05	0.15	0.33	0.748	23
ASD															
males	40.08	4.19	9.60	<0.001	95	0.32	0.09	3.81	<0.001	95	0.10	0.05	1.96	0.053	90
females	25.01	6.62	3.78	<0.001	68	0.67	0.13	5.10	<0.001	68	-7.52	0.08	-0.001	0.999	61

4.11 Figures

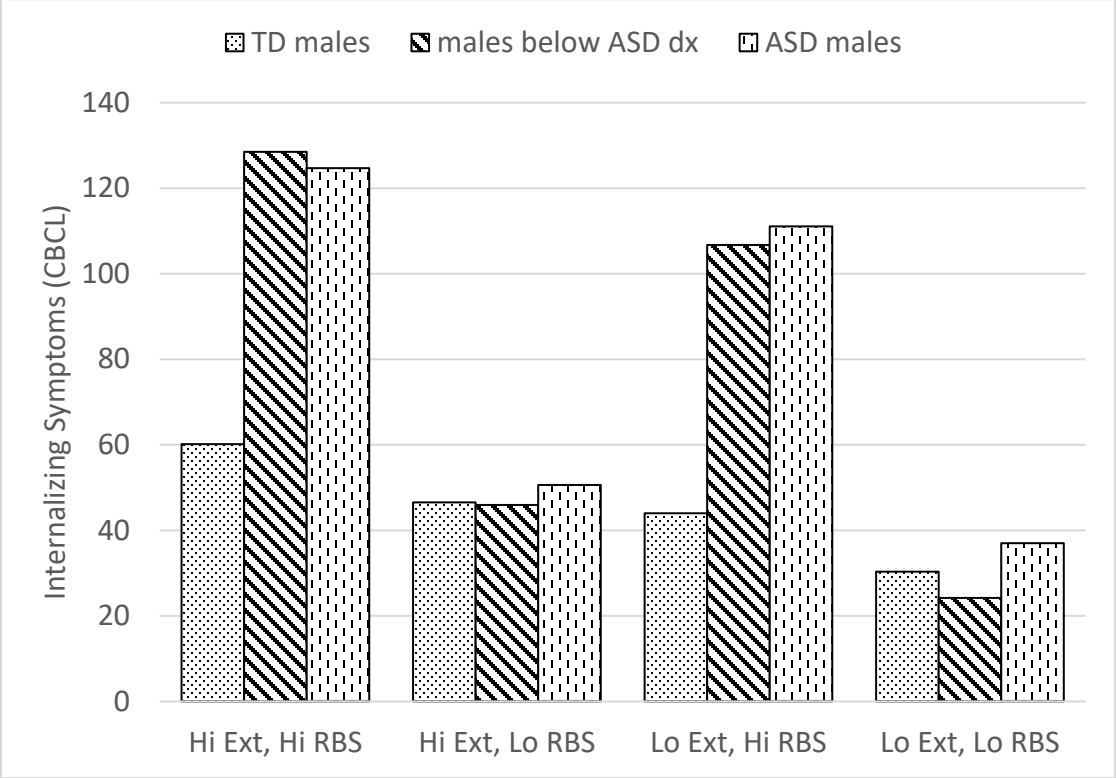

Figure 4.11.1 Externalizing and repetitive behaviors were associated with internalizing symptoms in TD males, males below ASD dx cutoff, and ASD male adolescents. Externalizing symptoms (Ext) were based off of the CBCL total scores. Repetitive behaviors (RBS) were based off of the Repetitive Behavior Scale – Revised total scores. Hi = +2SD from the mean indicating high externalizing and high repetitive behaviors. Lo = -2SD from the mean indicating low externalizing and low repetitive behaviors. Standard deviations (SD) were calculated within group means (TD, below ASD dx, ASD) to capture deviations that were standard for each group identifier.

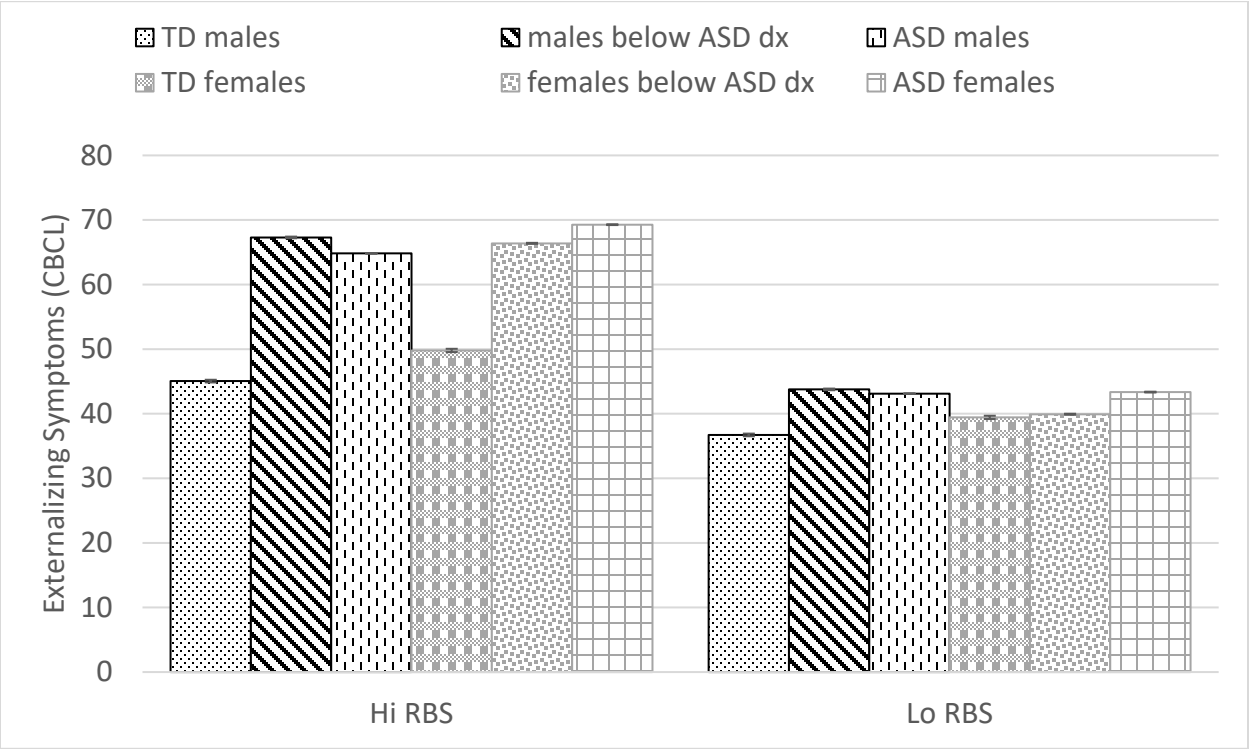

Figure 4.11.2 Repetitive behaviors (RBS) predict externalizing symptoms autistic adolescents. Hi = +2SD from the mean indicating high repetitive behaviors. Lo = -2SD from the mean indicating low repetitive behaviors. Standard deviations (SD) were calculated within gender and within group means (TD, below ASD dx, ASD females; TD, below ASD dx, ASD males) to capture deviations that were standard for each group identifier.

Supplemental

Table 4.A

	ASD						Below ASD dx cutoff						TD					
	female			male			female			male			female			male		
Measures	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N
ADOS	10.73	3.65	67	12.61	4.09	96	3.14	1.57	7	4.60	2.07	5	-	-	-	-	-	-
SRS	74.87	17.60	70	71.01	16.11	99	71.68	12.70	31	68.19	10.09	21	43.74	8.59	104	42.67	4.28	100
SCQ	16.81	6.64	69	19.43	7.38	101	13.57	9.88	30	12.38	8.16	21	1.83	3.40	109	2.47	4.28	102
RBS - R	23.43	18.25	63	23.24	18.53	94	17.37	20.48	27	19.75	20.64	16	1.98	3.57	93	1.60	3.41	91
CBCL - internalizing	62.99	11.41	68	59.74	8.49	95	59.00	12.49	26	61.05	11.65	19	47.84	10.29	107	44.04	9.02	97
CBCL - externalizing	56.31	10.84	68	53.93	10.55	95	53.15	9.92	26	55.53	10.58	19	44.45	9.21	107	40.89	6.90	97
CASI - ODD	2.59	2.76	64	2.20	2.51	89	1.42	1.98	19	1.76	2.36	17	0.30	0.99	97	0.30	1.04	91
CASI - CD	0.66	0.98	64	0.55	0.94	89	0.21	0.42	19	0.47	0.72	17	0.09	0.34	97	0.09	0.41	91
CASI - GAD	3.00	2.49	64	2.28	2.17	89	2.50	2.94	18	1.35	1.90	17	0.46	1.02	97	0.24	1.00	87
CASI - depression	0.91	1.08	64	0.53	0.85	89	0.58	0.92	19	0.50	0.92	17	0.09	0.37	97	0.12	0.59	91
PDSS (youth-reported)	2.84	1.10	51	2.61	1.06	78	3.73	1.14	22	2.87	1.02	15	3.14	1.19	86	2.88	1.15	84
PDSS (parent-reported)	2.88	1.12	57	2.27	1.33	76	3.55	1.24	22	2.87	1.27	15	2.74	1.32	74	2.47	1.18	71
Vineland - communication	41.79	19.90	70	36.61	16.00	102	61.04	27.07	28	55.41	22.13	17	55.80	23.46	107	52.79	22.78	102
Vineland - living skills	42.03	17.52	70	36.97	16.41	102	60.50	29.05	28	56.47	25.88	17	55.03	21.18	107	51.73	20.77	102

Note: Total scores are reported for all measures with the exception of the PDSS. Total scores represent symptom count for each measure. The Vineland was used for measures of verbal ability and adaptive ability. ADOS = Autism Diagnostic Observation Schedule. SRS = Social Responsive Scale. SCQ = Social Communication Questionnaire. RBS - R = Repetitive Behavior Scale - Revised. CBCL = Child Behavior Checklist. CASI = Child and Adolescent Symptom Inventory. PDSS = Pubertal Development Scale - Shirtcliff scale conversion.

CHAPTER 5. PUBERTAL DEVELOPMENT AND REPETITIVE BEHAVIOR PREDICTED DEPRESSIVE SYMPTOMS IN AUTISTIC ADOLESCENT MALES

Jenny M. Phan,[1] Katie Jankowski,[2] Elizabeth A. Shirtcliff,[1] & Jennifer Pfiefer,[2]

5.1 Author Contributions

Jenny Phan completed the writing and statistical analysis of the present study. Coauthors provided statistics consultation, reviewed, and edited drafts of the manuscript. Katie Jankowski was the principal investigator of the parent investigation through an Autism Speaks Weatherstone Predoctoral Fellowship

5.2 Abstract

The aim of the present study was to examine pubertal and behavioral effects on depressive symptoms between typically developing and autistic male adolescents between ages 11-17 years. Pubertal status and testosterone were investigated as markers of puberty and as predictors of depressive symptoms. Social responsiveness and restricted and repetitive behaviors were also tested as predictors of depressive symptoms. Findings showed autistic male adolescents had higher depressive symptoms than typically developing male adolescents. Pubertal status mapping onto adrenal development predicted elevated depressive symptoms for autistic males. Restricted and repetitive behaviors showed decreasing depressive symptoms for autistic male adolescents. Overall, group differences were found, and results on puberty and repetitive behaviors aligned with previous studies and the two-hit model of autism.

---

[1] Iowa State University, Department of Human Development and Family Studies, Ames, Iowa

[2] University of Oregon, Center for Translational Neuroscience, Eugene, Oregon

5.3 Introduction

Emerging adolescents go through a sensitive period of development that entails physical changes and endogenous influx of hormones intermediating physical and emotional development (Shirtcliff et al., 2009). The pubertal process, though a normative developmental milestone, is experienced differently for some youths relative to their peers. These differential pubertal experiences can vary due to risk factors that may increase vulnerability for some adolescents in developing internalizing symptoms (e.g., depression), and underlying neurobiological effects can contribute to added difficulties (Graber, 2013; Sisk & Zehr, 2005; Picci & Scherf, 2015).

Our study focused on males because depressive symptoms, such as depressed mood and anhedonia, affect some male adolescents as they transition through adolescence (Bennett, Ambrosini, Kudes, Metz, & Rabinovich, 2005), and since depression is more common in females, less attention is placed on depression in males. Adolescents with neurodevelopmental disorders, such as Autism Spectrum Disorder, are at a higher risk of experiencing depression (Magnuson & Constantino, 2011; De-la-Iglesia & Olivar, 2015) than typically developing youths. Comorbidity of autism and depression are compounding psychosocial factors contributing to added difficulties for autistic adolescents. Depressive symptoms may be overlooked in male adolescents, and in particular autistic male adolescents, due to a higher comorbidity of other psychiatric disorders masking internalizing problems. This, in turn, can invalidate internalizing problems for autistic male adolescents and draw attention more towards other comorbidities (i.e., externalizing behaviors; Magnuson & Constantino, 2011).

Presently, it is unclear how pubertal maturation affects autistic male adolescents and risk for comorbidity of depressive symptoms. Translational neuroscience research theorized a “double hit” of early neural circuity disturbances during prenatal development (Picci & Scherf, 2015). The first hit is when sex developmental hormones (e.g., testosterone) modulate neural

networks and lead to manifestations of altered brain development. The second hit is during pubertal development when the same sex developmental systems [i.e., hypothalamic-pituitary-adrenal axis (HPA), hypothalamic-pituitary-gonadal axis (HPG)] are re-activated resulting in physical changes (Schulz et al., 2009). Activation of the HPA and HPG axes during pubertal maturation releases sex hormones responsible for secondary sexual characteristics development; for example, growth in size of the testes which manufactures testosterone in males (Sato, Schulz, Sisk, & Wood, 2008). Byproducts of pubertal development may be typical for all adolescents undergoing puberty, and for autistic male adolescents who have comorbid depressive symptoms, these underlying physical and neurobiological changes can interact with psychosocial factors impacting social responsiveness.

Autistic individuals differ in social responsiveness relative to typically developing individuals. A widely used measure of social responsiveness for the autism population is the Social Responsiveness Scale (SRS), which assesses social categories, such as awareness, information processing, reciprocity, anxiety or avoidance (Constantino, 2013). Peer socialization is an important part of adolescent development, and social responsiveness is considered an important function in navigating interactions with peers. Social responsiveness may be a key autism feature to investigate further in its association with depressive symptoms in autistic male adolescents.

Few studies exist on repetitive behaviors, a core feature in autism, and its multidimensional function. Repetitive behavior could potentially be a form of coping with depression for autistic males; however, research examining repetitive behavior separate from other autism features are less common. A first step in understanding the function of repetitive behavior in depression is to test whether depressive symptoms increase or decrease as a function

of higher engagement in repetitive behaviors. A second step is to investigate whether repetitive behavior is also a feature in typically developing males as well as its association with depressive symptoms and whether these associations differ from those on the autism spectrum. Whether autistic adolescents find repetitive behaviors a form of coping with depression is still unclear.

This study aimed to investigate the associations among puberty, the pubertal hormone testosterone, repetitive behaviors, social responsiveness, and depressive symptoms, in typically developing and autistic male adolescents. The inclusion of males only allows us to control for sex as a biological variable in the study design. To date, no empirical literature has focused on pubertal maturation, restricted and repetitive behaviors, and social responsiveness as mechanisms for risks or protective effects on depressive symptoms in autistic adolescent males and then compare to typically developing males. Observations of behaviors during pubertal maturation in autistic adolescents has potential implications for understanding when and how some autistic adolescent males begin showing depressive symptoms and to test whether autism is a risk factor for depression when comparing to typically developing adolescent males.

We hypothesized autistic male adolescents would show more elevated depressive symptoms than typically developing male adolescents. We hypothesized that youths with higher pubertal status scores mapping onto adrenal and gonadal development, show more elevated depressive symptoms for autistic than typically developing male adolescents. We hypothesized deficits in social responsiveness skills predict elevated depressive symptoms, and that increases in restricted and repetitive behaviors would be associated with decreasing depressive symptoms in autistic youths compared to typically developing male youths.

5.4 Methods

Research participants included 74 adolescent males (31 autistic [ASD], 43 typically developing [TD]) between the ages of 11 to 17 years. Adolescent males in the autism group were

mean age 14.66 years, and typically developing (TD) adolescent males were mean age 14.83 years. The overall sample were predominantly Caucasian white. Race and ethnicity for the autism group included 89% (75% for TD group) were Caucasian white, 4% were Hispanic (17% for TD group), and 7% were multiethnic (8% for TD group). Exclusionary criteria were any individuals who reported any neurological disorder. Other exclusionary criteria were any psychiatric and/or neurodevelopmental disorder diagnosis for typically developing males. For autistic males, those who had a diagnosis of schizophrenia, Tourette's syndrome, dyslexia, intellectual disability, attention deficit hyperactivity disorder, depression, or anxiety disorder were excluded from the study. An IQ cutoff of greater than or equal to 75 on the FSIQ subscale of the WASI-II (McCrimmon & Smith, 2013) also determined inclusion criteria for this study. Adolescents in the autism group had a mean FSIQ of 109.33 and in the typically developing group a mean score of 116.56. The Autism Diagnostic Observation Schedule (Lord et al., 2012), the Social Responsiveness Scale (Constantino, 2005), the Autism Spectrum Rating Scale (Goldstein & Naglieri, 2012), and the Diagnostic Statistical Manual for Mental Health Disorders (DSM-5; American Psychiatric Association, 2013) were used to confirm adolescent males who met diagnostic criteria for autism spectrum disorder (see Table 5.8.1).

5.4.1 Measures

5.4.1.1 Restricted and Repetitive Behaviors

Total score of the subscale *stereotyped behaviors and restricted interests* of the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2012) was included in the present study as a measure of restricted and repetitive behaviors. Autistic adolescents had a mean score of 1.68 (SD = 1.11), and TD adolescents had a mean score of 1.33 (SD = 1.16). The scoring scale was 0 to 3.

5.4.1.2 Social Responsiveness

The Social Responsiveness Scale (Constantino, 2005) quantitatively measures social abilities in children and adolescents ages from 4 to 18 years. Autistic adolescents had a mean score of 102.72 (SD = 23.78, range 49-145), and for the TD adolescents, they had a mean score of 47.45 (SD = 39.28, range 6-131).

5.4.1.3 Depressive Symptoms

The Center for Epidemiologic Studies Depression Scale is a valid, reliable, and widely used measure of depressive symptoms for children and adolescents (Weissman, Orvaschel, & Padian, 1980). Autistic adolescents had a mean score of 0.88 (SD = 0.50, range 0.15-2.15). TD adolescents had a mean score of 0.63 (SD = 0.42, range 0-1.75).

5.4.1.4 Puberty

Self-reports of adolescent's sex-specific pubertal maturation were provided through the Pubertal Development Scale (PDS; Petersen et al., 1988). Participants reported changes in height, facial hair growth, body hair growth, skin changes, and voice deepening. A question on their perceived growth and development relative to their peers was also in the PDS. The PDS metric was converted into Tanner stages that map onto adrenal and gonadal development, respective of adolescent's biological sex, using a SPSS syntax developed by Shirtcliff and colleagues (2009). Tanner stages that map onto adrenarcheal development is largely driven by tertiary hormones such as dehydroepiandrosterone (DHEA) and its sulphate form DHEA-S, which typically begins around Tanner stage 1-2 (Guran et al., 2015); however, individual differences in adrenarcheal development has been shown, particularly at later Tanner stages (Netherton, Goodyer, Tamplin, & Herbert, 2004). Tanner stages that map onto gonadal development, again, respective of biological sex, mean that adolescents whose physical

maturation reported from the PDS aligns with biological changes that occur “under the skin,” such as hormones that drive changes to the gonads. In males, testosterone predominantly drive gonadal development across maturation. The PDS conversion scale to Tanner stages derived two sets of scores: PDSA (Tanner stages mapping onto adrenal development) and PDSG (Tanner stages mapping onto gonadal development). Autistic adolescents’ average PDSA (Tanner) stage were 3.65 (SD = 1.08), and TD adolescents’ average PDSA (Tanner) stage were 3.33 (SD = 1.17). The PDSG stage average for autistic adolescents were 2.98 (SD = 1.08) and for TD adolescents, they were mean Tanner stage 3.28 (SD = 0.97).

One saliva sample was collected in a microvial from each adolescent via passive drool, stored at -80 degrees Celsius, and shipped on dry ice to the Stress Physiology Investigative Team (SPIT) lab at Iowa State University for enzyme immunoassays. Saliva samples were thawed and assayed using Salimetrics kits (Salimetrics, LLC.) for testosterone. Funding for this study was limited, and saliva samples were only assayed for one analyte. A total of 28 saliva samples (for autistic youth) and 27 saliva samples (for typically developing youth) yielded assay results. Autistic youth’s mean testosterone level was 162.04 pg/mL (SD = 91.89, range 40.83—403.22) and was 138.54 pg/mL (SD = 61.77, range 19.56—246.13) for typically developing youth. No outliers were found. Testosterone data was skewed and therefore, was natural log transformed to normalize data.

5.5 Results

Multiple linear regressions were run to test group differences between autistic and TD adolescent males in depressive symptoms. Predictors were added to the regression models separately to examine the role of pubertal status (mapping onto gonadal development, mapping onto adrenal development, respectively), testosterone, social responsiveness, and restricted and repetitive behaviors on depressive symptoms. Grouping variable was coded 0 = TD adolescents

and 1 = autistic adolescents and controlled in every regression model. The variable PDSA represents pubertal status that mapped onto adrenal development, and the variable PDSG represents pubertal status that mapped onto gonadal development. Unstandardized beta coefficient, standard error, t-statistic, 95% confidence interval for beta, and p-value are reported with the results.

We examined correlations of the self-reported PDS adrenal and gonadal stages with testosterone. Testosterone was correlated with the PDS adrenal scores ($r = 0.692$) and with the PDS gonadal scores ($r = 0.552$).

First, we tested whether autistic adolescent males showed elevated depressive symptoms relative to TD males. A group difference in depressive symptoms was found ($b = 0.25$, $SE = 0.11$, $t = 2.32$, CI [0.035, 0.464], $p = 0.023$); autistic adolescent males showed elevated depressive symptoms compared to TD males.

Second, we investigated if pubertal status that mapped on adrenal and gonadal development (respectively) had an effect on depressive symptoms in males. PDSA showed a trend effect on depressive symptoms ($b = 0.091$, $SE = 0.05$, $t = 1.94$, CI [-0.002,0.184], $p = 0.056$), while controlling for group ($p = 0.042$ vs. $p = 0.023$). A deeper dive into these differences by splitting data by group and testing PDSA as a predictor of depressive symptoms showed a significant effect of PDSA on depressive symptoms for autistic males ($b = 0.244$, $SE = 0.08$, $t = 2.90$, CI[0.066, 0.382], $p = 0.007$), while for TD males, PDSA did not predict depressive symptoms ($b = 0.006$, $SE = 0.06$, $t = 0.17$, CI[-0.103, 0.122], $p = 0.865$). PDSG did not significantly predict depressive symptoms for autistic and TD males ($p = 0.139$).

Third, we tested whether testosterone levels played a role in depressive symptoms. We first tested whether testosterone levels differed by group and did not find a significant difference

(t = -0.749, p = 0.458). Testosterone levels did not significantly predict elevated depressive symptoms in males (p = 0.211). We examined whether the effect of PDSA on depressive symptoms for autistic males persisted after controlling for testosterone and found that PDSA's effect on depressive symptoms persisted (b = 0.303, SE = 0.11, t = 2.83, CI[0.082, 0.524], p = 0.009).

Fourth, we tested if social responsiveness and repetitive behaviors predicted depressive symptoms. Social responsiveness did not significantly predict depressive symptoms (p = 0.371). Repetitive behaviors predicted decreasing depressive symptoms (b = -0.173, SE = 0.07, t = -2.54, CI[-0.313, -.034], p = 0.017) when controlling for group, and this was apparent in autistic males (splitting data by group), b = -0.183, SE = 0.08, t = -2.32, CI[-0.344, -0.021], p = 0.028 but not for TD males (p = 0.74).

Lastly, we tested whether repetitive behaviors continued to predict decreasing depressive symptoms when controlling for PDSA. Repetitive behaviors continued to show decreasing depressive symptoms (b = -0.160, SE = 0.072, t = -2.23, CI[-0.306, -0.013], p = 0.034). The effect of PDSA was no longer significant when controlled in this regression model (p = 0.492).

5.6 Discussion

The present study investigated four aims in comparing between typically developing and autistic male adolescents. The first aim was to investigate if autistic males had higher depressive symptoms than typically developing males. The second aim was to investigate if pubertal development had an effect on depressive symptoms in male adolescents. The third aim was to investigate repetitive behavior and social responsiveness effect on depressive symptoms. The fourth aim was to test whether the pubertal hormone testosterone had an effect on depressive symptoms in male adolescents with and without autism.

Overall, a group difference in depressive symptoms was found between autistic and typically developing male adolescents. As hypothesized, autistic males showed higher depressive symptoms than typically developing males. A closer examination into group effects consistently showed that variations in depressive symptoms were apparent in autistic males but not by typically developing males. Emerging research are finding a growing prevalence of depression among the autism population and that their experiences with depression are not all that similar to typically developing individuals (Gadow, Guttmann-Steinmetz, Rieffe, & DeVincent, 2012). A study found links between feelings of depression and worthlessness when autistic adolescent males touched others unusually (Bitsika & Sharpley, 2016). For many autistic individuals, touch is a sensory stimulation that is often paired with social appropriateness that can have a negative connotation when an individual's autism status is unbeknownst to the person being touched. An additional challenge for autistic males is the consent dialogue. In some instances when consent is less clear, autistic males struggle with understanding social ambiguities that most other typically developing males better understand (Mackenzie & Watts, 2013); all of which could potentially explain differences in depressive symptoms between typically developing and autistic male adolescents. Bitsika and Sharpley (2016) also found that pre-adolescent males felt depressed, worthlessness, and fatigue and that these feelings were associated with lack of self-confidence in social interactions. Another study found repetitive cognition, also known as rumination, covaried with autism spectrum symptomatology, has an association with depression (Keenan, Gotham, & Lerner, 2018). It is worth noting that rumination leading to depressive symptoms is not unique to autistic individuals (McLaughlin et al., 2014). Rumination may be a risk factor of depression for adolescent males, and perhaps, an important consideration is that depressive symptom

presentation or manifestation in males compared to females (Khesht-Masjedi et al., 2017) may be misdiagnosed for other disorders (Aggarwal & Angus, 2015).

We tested the two-hit model of autism (Picci & Scherf, 2015) by investigating two pubertal stage scores that mapped onto the development of the adrenal cortex and the testes in males. Secondary sexual characteristics changes during puberty are associated with the adrenal cortex and testes development, and each of these organs serve different functions. An adrenal puberty score and a gonadal puberty score were developed to capture these different aspects of physical maturation. In the present study, the adrenal puberty score predicted elevated depressive symptoms for autistic males but not for the gonadal puberty score. This indicated that autistic males who were more developed in puberty by adrenal maturation had an impact on depressive symptoms elevation. Pubertal development that map onto adrenal development also captures the social implications related to physical development, such as social stigma of increasing acne and body odor (Bhate & Williams, 2013; Scott & Walsh, 2014). A current gap in the research literature is on the social implications of pubertal development for autistic males. The present study contributes to the dearth of literature on pubertal maturation impact on development of internalizing symptoms in autistic males. Though the PDSA was an indirect measure of secondary sexual characteristics maturation during puberty, the result of the PDSA predicting elevated depressive symptoms for autistic males was in line with the two-hit model of autism.

Repetitive behavior is one key feature of autism and even typically developing adolescents engage in repetitive behaviors, as was shown in the present study. The function of repetitive behaviors in the context of increasing internalizing problems are less understood, except that a positive association between repetitive behaviors and internalizing problems was previously found in several studies (Stratis & Lecavalier, 2013; Chebli, Martin, & Lanovaz,

2016; Worley & Matson, 2011; Antezana et al., 2019). Studies on repetitive behavior and depressive symptoms during adolescence with a focus on male adolescents are limited. As hypothesized, this study found repetitive behavior to lower depressive symptoms in autistic males. Perhaps, some aspects of repetitive behaviors can be adaptive, especially for autistic individuals, in that a commonly used behavior can function as a source of comfort when under distress or feeling depressed (Manor-Binyamini & Schreiber-Divon, 2019; Li et al., 2018). There are aspects of repetitive behaviors that may be more maladaptive, such as self-injurious behaviors (SIB), and SIB has often been found linked to internalizing problems (Stratis & Lecavalier, 2013; Antezana et al., 2019). More research is needed to study various aspects of repetitive behaviors and its functioning as either a protective or exacerbator of internalizing symptoms for autistic males as well as typically developing males.

Contrary to hypothesis, social responsiveness did not have an effect on depressive symptoms in male adolescents. As mentioned above, Bitsika and Sharpley (2016) found an association between social responsiveness and depression in male adolescents. With very few studies on social responsiveness and depression in male adolescents, it is difficult to generalize these findings and the mixed results between the present study and Bitsika and Sharpley (2016).

This study's unique contribution was to test testosterone as a neurobiological marker of puberty within the framework of the two-hit model of autism. Testosterone did not significantly predict elevated depressive symptoms. Pubertal maturation that maps onto gonadal development also did not predict depressive symptoms. Testosterone and gonadal development during puberty were unlikely mechanisms in predicting depressive symptoms. This then points to adrenal maturation and adrenal androgens as a potential mechanism in better understanding the two-hit model of autism. Though testosterone did not predict depressive symptoms in this study,

previous studies found testosterone to be associated with externalizing symptoms (Maras et al., 2003; Grotzinger et al., 2018). With that said, testosterone's role in the two-hit model of autism should not be discounted yet. Another underexplored research area is the association between high testosterone level and heterotypic comorbidity in autism. A prospective study using umbilical cord blood testosterone found associations with both internalizing and externalizing symptoms in males ages 2 to 10 years across development (Robinson et al., 2013); however, it is still unclear as to whether testosterone is associated with both symptoms type and severity during puberty. Future research may need to focus attention on testosterone's role in comorbidities during puberty with autism.

5.7 Limitations

Several limitations are noted. The sample size was small, and therefore, results from this study may not be generalizable for male adolescents. With the focus of the present study on male adolescents, however, it allowed for a deeper dive into the effects of autistic behaviors and normative developmental factors on depressive symptoms that are male-specific. Another limitation of the present study was that we only examined testosterone as a pubertal hormone. Previous studies have found other androgens, such as DHEA, a neurosteroid, to be linked to depression in adolescent males (Goodyer et al., 2000). Future research may need to include DHEA alongside testosterone as potential biomarkers of the two-hit model of autism during puberty. The present study only examined depressive symptoms in association with repetitive behavior and social responsiveness. Other psychopathology symptoms may contribute to a better understanding of the two-hit model of autism, and therefore, future research should consider externalizing symptoms as well as internalizing symptoms in the model.

References

Aggarwal, S., & Angus, B. (2015). Misdiagnosis versus missed diagnosis: Diagnosing autism spectrum disorder in adolescents. *Australasian Psychiatry*. https://doi.org/10.1177/1039856214568214

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders*. https://doi.org/10.1176/appi.books.9780890425596

Antezana, L., Factor, R. S., Condy, E. E., Strege, M. V., Scarpa, A., & Richey, J. A. (2019). Gender differences in restricted and repetitive behaviors and interests in youth with autism. *Autism Research*, *12*(2), 274–283. https://doi.org/10.1002/aur.2049

Bennett, D. S., Ambrosini, P. J., Kudes, D., Metz, C., & Rabinovich, H. (2005). Gender differences in adolescent depression: Do symptoms differ for boys and girls? *Journal of Affective Disorders*, *89*(1–3), 35–44. https://doi.org/10.1016/j.jad.2005.05.020

Bhate, K., & Williams, H. C. (2013). Epidemiology of acne vulgaris. *British Journal of Dermatology*. https://doi.org/10.1111/bjd.12149

Bitsika, V., & Sharpley, C. F. (2015). Differences in the Prevalence, Severity and Symptom Profiles of Depression in Boys and Adolescents with an Autism Spectrum Disorder versus Normally Developing Controls. *International Journal of Disability, Development and Education*. https://doi.org/10.1080/1034912X.2014.998179

Chebli, S. S., Martin, V., & Lanovaz, M. J. (2016). Prevalence of Stereotypy in Individuals with Developmental Disabilities: a Systematic Review. *Review Journal of Autism and Developmental Disorders*, *3*(2), 107–118. https://doi.org/10.1007/s40489-016-0069-x

Constantino, J.N. (2005). *Social Responsiveness Scale*. Retrieved from https://www.wpspublish.com/store/p/2993/srs-social-responsiveness-scale

Constantino, J. N. (2013). Social Responsiveness Scale. In *Encyclopedia of Autism Spectrum Disorders*. https://doi.org/10.1007/978-1-4419-1698-3_296

De-la-Iglesia, M., & Olivar, J.-S. (2015). Risk Factors for Depression in Children and Adolescents with High Functioning Autism Spectrum Disorders. *The Scientific World Journal*, *2015*, 1–17. https://doi.org/10.1155/2015/127853

Gadow, K. D., Guttmann-Steinmetz, S., Rieffe, C., & DeVincent, C. J. (2012). Depression Symptoms in Boys with Autism Spectrum Disorder and Comparison Samples. *Journal of Autism and Developmental Disorders*, *42*(7), 1353–1363. https://doi.org/10.1007/s10803-011-1367-x

Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.177.6.499

Goldstein, S., & Naglieri, J. A. (2012). *Autism Spectrum Rating Scales*. Retrieved from https://www.acer.org/files/ASRS-Tech-Supp.pdf

Graber, J. A. (2013). Pubertal timing and the development of psychopathology in adolescence and beyond. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2013.04.003

Grotzinger, A. D., Mann, F. D., Patterson, M. W., Tackett, J. L., Tucker-Drob, E. M., & Harden, K. P. (2018). Hair and Salivary Testosterone, Hair Cortisol, and Externalizing Behaviors in Adolescents. *Psychological Science*, *29*(5), 688–699. https://doi.org/10.1177/0956797617742981

Guran, T., Firat, I., Yildiz, F., Kaplan Bulut, I., Dogru, M., & Bereket, A. (2015). Reference values for serum dehydroepiandrosterone-sulphate in healthy children and adolescents with emphasis on the age of adrenarche and pubarche. *Clinical Endocrinology*. https://doi.org/10.1111/cen.12612

Keenan, E. G., Gotham, K., & Lerner, M. D. (2018). Hooked on a feeling: Repetitive cognition and internalizing symptomatology in relation to autism spectrum symptomatology. *Autism*, *22*(7), 814–824. https://doi.org/10.1177/1362361317709603

Khesht-Masjedi, M., Shokrgozar, S., Abdollahi, E., Golshahi, M., & Sharif-Ghaziani, Z. (2017). Comparing depressive symptoms in teenage boys and girls. *Journal of Family Medicine and Primary Care*. https://doi.org/10.4103/jfmpc.jfmpc_129_17

Li, T., Hu, Y., Song, C., Lu, H., & Yi, L. (2018). The measurements and mechanisms of restricted and repetitive behaviors in autism spectrum disorders. *Kexue Tongbao/Chinese Science Bulletin*. https://doi.org/10.1360/N972017-01249

Lord, C., Rutter, M., DiLavore, P., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism Diagnostic Observation Schedule* (2nd ed.). Retrieved from https://www.wpspublish.com/store/p/2648/ados-2-autism-diagnostic-observation-schedule-second-edition

Netherton, C., Goodyer, I., Tamplin, A., & Herbert, J. (2004). Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology*. https://doi.org/10.1016/S0306-4530(02)00150-6

Mackenzie, R., & Watts, J. (2013). Sexual health, neurodiversity and capacity to consent to sex. *Tizard Learning Disability Review*, *18*(2), 88–98. https://doi.org/10.1108/13595471311315119

Magnuson, K. M., & Constantino, J. N. (2011). Characterization of Depression in Children With Autism Spectrum Disorders. *Journal of Developmental & Behavioral Pediatrics*, *32*(4), 332–340. https://doi.org/10.1097/DBP.0b013e318213f56c

Manor-Binyamini, I., & Schreiber-Divon, M. (2019). Repetitive behaviors: Listening to the voice of people with high-functioning autism spectrum disorder. *Research in Autism Spectrum Disorders*. https://doi.org/10.1016/j.rasd.2019.04.001

Maras, A., Laucht, M., Gerdes, D., Wilhelm, C., Lewicka, S., Haack, D., … Schmidt, M. H. (2003). Association of testosterone and dihydrotestosterone with externalizing behavior in adolescent boys and girls. *Psychoneuroendocrinology*. https://doi.org/10.1016/S0306-4530(02)00119-1

McCrimmon, A. W., & Smith, A. D. (2013). Review of the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II) . *Journal of Psychoeducational Assessment*. https://doi.org/10.1177/0734282912467756

McLaughlin, K. A., Aldao, A., Wisco, B. E., & Hilt, L. M. (2014). Rumination as a transdiagnostic factor underlying transitions between internalizing symptoms and aggressive behavior in early adolescents. *Journal of Abnormal Psychology*. https://doi.org/10.1037/a0035358

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence*, *17*(2), 117–133. https://doi.org/10.1007/BF01537962

Picci, G., & Scherf, K. S. (2015). A Two-Hit Model of Autism: Adolescence as the Second Hit. *Clinical Psychological Science*, *3*(3), 349–371. https://doi.org/10.1177/2167702614540646

Robinson, M., Whitehouse, A. J. O., Jacoby, P., Mattes, E., Sawyer, M. G., Keelan, J. A., & Hickey, M. (2013). Umbilical Cord Blood Testosterone and Childhood Internalizing and Externalizing Behavior: A Prospective Study. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0059991

Salimetrics, LLC. (n.d.). Retrieved from https://www.salimetrics.com/

Sato, S. M., Schulz, K. M., Sisk, C. L., & Wood, R. I. (2008). Adolescents and androgens, receptors and rewards. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2008.01.010

Schulz, K. M., Molenda-Figueira, H. A., & Sisk, C. L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior*, *55*(5), 597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

Scott, S. M., & Walsh, A. M. (2014). Adolescent Sexual Development: An Overview of Recent Research. *Canadian Journal of Community Mental Health*, *33*(1), 21–29. https://doi.org/10.7870/cjcmh-2014-003

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337. https://doi.org/10.1111/j.1467-8624.2009.01263.x

Sisk, C. L., & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*, *26*(3–4), 163–174. https://doi.org/10.1016/j.yfrne.2005.10.003

Stratis, E. A., & Lecavalier, L. (2013). Restricted and repetitive behaviors and psychiatric symptoms in youth with autism spectrum disorders. *Research in Autism Spectrum Disorders*, *7*(6), 757–766. https://doi.org/10.1016/j.rasd.2013.02.017

Weissman, M. M., Orvaschel, H., & Padian, N. (1980). Children's symptom and social functioning self-report scales. Comparison of mothers' and children's reports. *The Journal of Nervous and Mental Disease*, *168*(12), 736–740. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7452212

Worley, J. A., & Matson, J. L. (2011). Psychiatric symptoms in children diagnosed with an Autism Spectrum Disorder: An examination of gender differences. *Research in Autism Spectrum Disorders*, *5*(3), 1086–1091. https://doi.org/10.1016/j.rasd.2010.12.002

5.8 Tables

5.8.1 Scores Qualified for a Clinical Cutoff for an ASD *dx*

Measures	Scores
Autism Diagnostic Observation Schedule	7+
Social Responsiveness Scale	55+
Autism Spectrum Rating Scale	60+
Diagnostic Statistical Manual for Mental Health Disorders	60+

Note: For the ADOS, the combined scores for social and communication domains determined cutoff. Other cutoff scores were total scores.

CHAPTER 6. GENERAL DISCUSSION

The goals of the studies in Chapters 3, 4, and 5 were to examine associations between puberty and internalizing symptoms that tend to be overlooked in males, to disentangle comorbid symptoms of internalizing and autistic behaviors in adolescent males, and to determine best fitting neurobiological models that are developmentally sensitive within autistic male adolescents. Each of these studies offer insights into the mechanisms lending information on both risk and normative factors that explain symptoms severity within youth who are typically developing and neurodevelopmentally different.

Within- and between-males developmental trajectory of symptoms severity and directionality. Chapter 3 offered a rare glimpse into the developmental trajectories of psychopathology symptoms in male youths transitioning through physical maturation and chronological age. I investigated puberty as a predictor of psychopathology symptoms and directionality across developmental trajectory in child and adolescent males. Growth curve models combined with an accelerated longitudinal design was utilized to examine processes of symptoms severity and directionality stability or fluctuations over time. Age was a consistent theme in predicting both symptoms severity and directionality in which older youths had more elevated severity of symptoms and had a preponderance of internalizing symptoms. Conversely, for younger youths, they showed a preponderance of externalizing symptoms. Male adolescent's chronological age was found to be a predictor of symptoms elevation, and this was apparent for male adolescents who already transitioned from child development into adolescent development.

Pubertal timing was also a normative predictor of symptoms severity but not for symptom directionality. Pubertal timing did not explain any variance of symptom directionality but contributed to the predictive validity of age effect on directionality. However, pubertal

timing contributed to explaining the variance of symptom severity in that early developers had greater symptoms severity than average and late developers.

Pubertal hormones also contributed to explaining symptoms severity and directionality, which have normative neurobiological impact on physical changes as youth undergo puberty. Hormonal impacts on psychopathology symptoms are somewhat more complex but are observable. DHEA and testosterone levels had opposite effects on symptoms severity, specifically, youth with higher DHEA levels had lower symptoms severity; youth with higher testosterone levels had elevated symptoms severity. High testosterone levels also predicted a preponderance of externalizing symptoms. Pubertal timing and pubertal hormones uniquely contribute to explaining symptoms severity and directionality developmental trajectory for male youths as well as explained some of the individual differences between males. In summary, findings from that study support the idea of development is complex and that it is important to capture normative processes in investigations of psychopathology symptoms severity and directionality when studying adolescent males.

The links between repetitive behaviors and heterotypic comorbidity of internalizing and externalizing symptoms. Chapter 4 aimed to examine the associations among repetitive behavior, externalizing symptoms, and internalizing symptoms, and the role of puberty in those associations in typically developing and autistic adolescents. A total of six groups of adolescents were included: males and females who were typically developing, below cutoff for an ASD diagnosis, and autistic. As hypothesized and aligned with previous research, autistic adolescents had higher repetitive behavior, externalizing symptoms, and internalizing symptoms than typically developing adolescents. Autistic adolescents also had higher oppositional defiant symptoms, conduct behavior symptoms, generalized anxiety symptoms, and depressive

symptoms than typically developing adolescents. Females had higher internalizing symptoms than males, and autistic females had higher generalized anxiety symptoms than all other groups. Other risk factors contributing to elevated internalizing symptoms for autistic adolescents were not tested in this study and would be essential to further examine in gaining knowledge as to why autistic adolescents are exhibiting higher internalizing symptoms than typically developing adolescents.

A novel contribution of this study to the research literature was the investigation of repetitive behavior along with heterotypic comorbidity. Surprisingly, repetitive behavior and externalizing symptoms each predicted elevated internalizing symptoms, and that these effects were specific to typically developing males and males who were below an ASD cutoff diagnosis. Not so surprisingly, typically developing male adolescents who were more developed pubertally had elevated internalizing symptoms, which was also found in Chapter 3 with elevated symptoms severity for early developers. Repetitive behavior also predicted elevated externalizing symptoms for males. However, another surprising finding was that autistic male adolescents who were more developed pubertally had decreasing externalizing symptoms. As could be expected, decreased externalizing symptoms was also found for autistic youth who had better verbal ability. With limited understanding on the impact of pubertal development on externalizing symptoms for autistic male adolescents, more research is needed to elucidate the role of pubertal maturation on behaviors for autistic adolescents. Put together, these findings indicate there are gender and group differences in repetitive behavior, externalizing, and internalizing symptoms and that for certain behavior/symptoms associations, those associations supersede gender and group effects, especially viewed through the lens of heterotypic comorbidity of externalizing and internalizing symptoms.

Physical maturation, neurobiological, and autistic stereotypy associations with depressive symptoms. In Chapter 5, I investigated pubertal development, the pubertal hormone testosterone, social responsiveness, and repetitive behavior as predictors of depressive symptoms in typically developing and autistic male adolescents. As hypothesized, autistic male adolescents had higher depressive symptoms than typically developing males. An underexplored but potentially important finding was that pubertal maturation that mapped onto adrenal development predicted elevated depressive symptoms for autistic males. Although this study did not examine social factors that may contribute to increasing depressive symptoms for males undergoing physical maturation, the finding of adrenal maturation mapped onto puberty fits with the two-hit model of autism. Future research is needed to identify those social factors or other puberty-related factors that explain increasing depressive symptoms for autistic males.

As hypothesized, repetitive behavior predicted decreasing depressive symptoms for autistic males. The multifunction of repetitive behavior in autism is still; however, typically developing adolescents also engage in repetitive behavior, as was also found in Chapter 4. Repetitive behavior may be a transdiagnostic factor of heterotypic comorbidity and certainly more research is needed to study repetitive behavior in typically developing and autistic adolescents.

In sum, developmental trajectories are generally stable, which is best ascribed to individual differences (e.g., age, pubertal timing, hormone profile). A cross-sectional examination of development may provide a small glimpse into individual differences in psychopathology symptoms severity. Both normative and risk factors are important to account when examining developmental trajectories of symptoms severity and directionality in typically developing and autistic adolescents. Gender-differentiated pathways is also an important

consideration in developmental models that combine neurobiological predictors of psychopathology symptoms. Heterotypic comorbidity is discovered to be more common and expected as youth transition through pubertal maturation. The future of developmental science needs to consider the codependency of developmental and neurobiological pathways in gaining a better understanding of heterotypic comorbidity in adolescence.

CHAPTER 7. GENERAL IMPLICATIONS

Two primary theoretical models were utilized to address 3 critical gaps in this dissertation research. The transdiagnostic model of psychopathology (TMP) and the two-hit model of autism guided this investigation of three critical gaps, specifically, male puberty and male-specific psychopathology preponderance as well as autistic male puberty and autistic-male-specific psychopathology preponderance are understudied. Additionally, biopsychosocial processes leading to mental health problems are not well understood. Findings from this dissertation in using the TMP further affirmed that heterotypic comorbidity is common in adolescence as was previously found in extant literature. The application of the TMP provides a snapshot into dimensional and dynamic symptom constructs that may wax and wane across development. It also allows researchers to examine when stability of symptoms as well as covariates that explain stability of symptoms across development. There are numerous biopsychosocial processes that can explain heterotypic comorbidity in adolescence, and in accounting for each of those processes, it has a great potential impact for better interventions or even preventions of mental health problems in adolescence.

Recent studies are breaking boundaries by expanding the transdiagnostic model of psychopathology using hard approaches to study comorbidities. These hard approaches mean that categorical psychopathology disorder constructs are broken down to subscales that formed those constructs. For example, the use of latent factor analyses that include multiple subscales (e.g., mood symptoms, overt aggression, anxiety symptoms, repetitive behavior) have potential explanation power as a latent factor predicting elevated depression. Such a psychopathology model must also consider developmental and gender-specific moderators that further explain variance components of elevated depression. Implications of this kind of research has potential

for targeting risk factors associated with specific comorbidities and either develop treatments or reform previous treatments to combat mental health problems for youth.

Why is male puberty and male-specific psychopathology preponderance understudied? Why will studying this topic impact mental health interventions and even prevention of mental illness in adolescence? This topic is likely understudied because previous research focused on gender differences between males and females. Female pubertal developmental process linked with onset of mental health problems is a dynamic and robust developmental model. When observing internalizing outcomes, many studies found female-preponderance of depression, mood, somatic, and anxiety problems. With so much attention on females, males tend to get overlooked in terms of internalizing outcomes and its association with pubertal maturation. This dissertation examined male-specific processes when observing internalizing outcomes and supported the need to examine normative risk factors (puberty, hormones) to better understand male-specific pathways that lead to mental health outcomes. Studies that examine gender differences are testing sex as a biological variable. An important consideration is to take a step further to investigate gender-specific outcomes and explore different pathways that might explain differential outcomes for males than for females. This is especially important for supporting youth undergoing puberty that may experience difficulty during this transitional period. An implication for this approach is to take a preventative intervention plan and design gender-sensitive treatments for better efficacy and prognosis.

Lastly but importantly, the two-hit model of autism paired with a dearth of empirical studies largely inspired this dissertation research. In this dissertation, results showed differential pubertal impact on psychopathology symptoms in youths. To make meaning of these results would require study replications and robust measures of puberty, which were limitations noted in

this dissertation. Processes that are better understood for typically developing youths may or may not apply for autistic youths. Additionally, there may be differential gender-specific pathways leading to mental health outcomes for autistic youths relative to TD youths. Once more research is underway, the field of autism and psychopathology interdisciplinary research will then be able to provide suggestions for preventative interventions. An implication of this kind of research could decrease the number of psychiatric admissions in hospitals for autistic individuals.

CHAPTER 8. GENERAL CONCLUSION

Heterotypic comorbidity was a common theme between Chapters 1 and 2 and is worth paying attention to in future research on male adolescent development. With a focus on male-specific observations of psychopathology, it was clear that males were as susceptible to internalizing symptoms as females. Puberty and pubertal hormones each uniquely contributed in explaining symptoms severity and directionality within male youth, and it was between-individual differences that explained some of those complexities. Due to this complexity, it is necessary for research to examine both puberty and pubertal hormones when studying gender-differentiated adolescent development. Autistic adolescents were at a higher risk of elevated internalizing and externalizing symptoms than typically developing adolescents, but that risk was not simplistically due to pubertal development. This was why an investigation of autistic stereotypy was needed to further understand behaviors beyond just development that was associated with psychopathology symptoms. Autistic stereotypy, such as restricted and repetitive behavior, and its association with psychopathology symptoms severity informs our understanding of behaviors that reveal similarities between typically developing and autistic youth. Another advantage in learning more about autistic stereotypy in both typically developing and autistic youth is to view those behaviors potentially as protective against increasing symptoms severity. Both risk and normative factors are important to consider in future research when studying community-based samples of typically developing and autistic youth. Those normative factors can also include behaviors that are key features commonly identified in autism.