



# Development and Parasympathetic Regulation in Male and Female Adolescents with Autism Spectrum Disorder: A Two-Timepoint Longitudinal Study

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## Abstract

Prolonged dysregulation of the autonomic nervous system (ANS) may increase propensity for physical or psychiatric illness. The current study examined differences in respiratory sinus arrhythmia (RSA) regulation in 215 adolescents with or without autism spectrum disorder (ASD) at Time 1 (T1; 10–13 years old) and 1 year later (Time 2; T2). Linear mixed effects models demonstrated lower RSA regulation in ASD, and a small interaction effect, showing blunted change in RSA from T1 to T2. Developmental differences in RSA regulation were particularly notable in females with ASD and those taking psychotropic medications. Results expand previous findings of reduced parasympathetic regulation in ASD by revealing a blunted developmental slope, indicating diagnostic differences may persist or worsen over time, particularly in females.

**Keywords** Autism · Parasympathetic · Respiratory sinus arrhythmia · Development · Sex

The parasympathetic nervous system (PNS), one of two branches of the autonomic nervous system (ANS), is involved in maintenance and regulation of unconscious, automatic bodily systems. The PNS regulates ‘rest and digest’ functions, such as lowering heart rate or blood pressure and increasing digestion. It largely serves to decrease physiological arousal, promote a state of relative calm, and conserve energy. In coordination with the sympathetic nervous system (SNS; ‘fight or flight’ branch), the PNS serves to regulate behavior and adaptive physiological response to changing environmental stimuli (Berntson et al., 2008). Consequences of proper or atypical functioning of the ANS thus go beyond physiology, as its moderating role in behavioral

and emotional regulation may have significant implications for a broad range of psychopathologies.

The activity of the PNS can be measured by observing changes in visceral organ function such as heart rate, as the autonomic system innervates the sinoatrial node—the pacemaker—of the heart (Hamill et al., 2012; Longhurst & Fu, 2012). A common marker of autonomic function, heart rate variability (HRV), utilizes changes in the beat-to-beat intervals in heart rate to inform the relative contributions of both the PNS and the SNS. For example, changes in HRV within high frequency respiration ranges (Respiratory sinus arrhythmia; RSA) indicate changes in PNS regulation. Pharmacological blockade studies have shown that RSA is sensitive to cholinergic, but not adrenergic, receptor blockade, providing empirical support for its use as a parasympathetic marker (Berntson et al., 1993; Cacioppo et al., 1994). Furthermore, an extensive, interconnected neural network is responsible for regulating autonomic function, extending from reflexive or stimulus-specific control at the spinal- and brainstem-level to more integrated behavioral responses requiring cognitive processing at the forebrain level (Benarroch, 2012). While several brainstem regions coordinate physiological response by processing afferent stimuli, (e.g., Hamill et al., 2012) several major forebrain regions, collectively known as the Central Autonomic Network (Benarroch, 1993), exert additional top-down control over the system. For example,

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the amygdala can modulate autonomic response to emotional stimuli through direct connections with brainstem regions (Schwaber et al., 1982). The prefrontal cortex, which coordinates a number of cognitive control functions, projects to other major limbic regions (Thayer & Lane, 2000), including the amygdala and hippocampus, thereby positioning itself at the top of the response hierarchy (Ulrich-Lai & Herman, 2009).

Autonomic dysregulation has been implicated in autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by impaired social communication and presence of restricted, repetitive behaviors and interests (APA, 2013). Current estimates suggest that one in every 54 children is diagnosed with ASD by the age of 8 years old (Maenner et al., 2020). Males are 4 times more likely to be diagnosed with ASD relative to females, although recent evidence suggests the ratio may be closer to 3:1 (Loomes et al., 2017). Individuals with ASD are at elevated risk for a number of comorbid conditions, including but not limited to epilepsy, sleep disorders, gastrointestinal dysfunction, attention deficit/hyperactivity disorder, anxiety disorders, and depression (e.g., Accardo & Malow, 2015; McElhanon et al., 2014; Simonoff et al., 2012). Comorbid rates of depression and anxiety are especially high, with lifetime anxiety prevalence recently estimated at 42% (Hollocks et al., 2019), and percentage of individuals diagnosed with a depressive disorder by adulthood estimated at 40% (Hollocks et al., 2019; Hudson et al., 2019), far surpassing global rates (Mayes et al., 2011). Notably, a dynamic equilibrium between the two branches of the ANS is said to be associated with fewer mental health problems (Nederhof et al., 2015). In contrast, an inflexible, invariable system is often characteristic of psychopathological states (Friedman, 2007; Friedman & Thayer, 1998; Thayer et al., 1996, 2000), highlighting the significance of identifying ANS dysfunction in autism and the extent to which it may contribute to psychopathologies in these individuals.

Previous research in ASD and ANS functioning often centers around the social deficits characteristic of the neurodevelopmental disorder, guided by the Polyvagal Theory (Porges, 1995, 2001, 2003a, 2007), which proposes that a parasympathetically-mediated default state regulates a ‘social engagement system’ (Porges, 2001, 2003b) of interconnected brainstem nuclei that regulate the myelinated vagus as well as cranial nerves directly involved in controlling muscles of the face and head. Therefore, in a parasympathetic-dominant system, calm visceral states may promote these pro-social behaviors such as eye contact and language production. However, if the PNS is poorly regulated and/or overridden by the SNS branch, this will block the social engagement system (Porges, 2003a, 2003b, 2007). Further, the opposite may be true, in which social difficulties contribute to autonomic dysfunction.

For example, social impairments characteristic of ASD may predispose youth to difficult peer interactions, and over time older youth with ASD may become more aware of these social challenges and the effects on relationships (Knott et al., 2006; Kuusikko et al., 2008; Lopata et al., 2008). This may in turn shape future social anxiety (Bellini, 2006), driving limbic system regions to over-activate the ‘fight or flight’ sympathetic response and decrease parasympathetic regulation. As such, dysfunction within the vagal and social engagement system could be bidirectional with socially adaptive behaviors, signaling significant social and physiological implications for a number of clinical populations, including ASD.

Indeed, decreased parasympathetic regulation has been reported in individuals with ASD relative to those with typical development (TD) (Bal et al., 2010; Edmiston et al., 2016; Guy et al., 2014; Ming et al., 2005; Neuhaus et al., 2016; Vaughan Van Hecke et al., 2009). Further evidence notes atypical autonomic response to stressors, including reduced vagal parasympathetic flexibility (Muscatello et al., 2021a, 2021b; Neuhaus et al., 2016; Schaaf et al., 2015; Vaughan Van Hecke et al., 2009) as well as an atypically blunted sympathetic response (Edmiston et al., 2017). Nevertheless, it should be mentioned that several studies cite no significant autonomic differences in ASD, either during resting state (Kushki et al., 2014; Levine et al., 2012; Muscatello et al., 2020; Watson et al., 2012) or in response to a stressor (Hollocks et al., 2014; Kushki et al., 2014; Sheinkopf et al., 2013).

The variability in findings is likely a reflection of the heterogenous presentation of ASD, as well as contributions of factors contributing to vagal and autonomic functioning, including physical health, (e.g., Masi et al., 2007; Molfino et al., 2009; Shibao & Okamoto, 2012; Thayer & Sternberg, 2006), age, pubertal development (e.g., Eyre et al., 2014; Hartevelde et al., 2021; Hinnant et al., 2011), and/or sex differences (e.g., Hartevelde et al., 2021; Hinnant et al., 2011; Koenig et al., 2017). For example, there is evidence that resting-state HRV increases progressively from young- to middle-childhood, before reaching a relative plateau in late adolescence (Eyre et al., 2014; Hartevelde et al., 2021), although stability may be affected by demographic factors such as race (Hinnant et al., 2011). For ASD, a recent study of autonomic reactivity during a social interaction task found that in school-aged youth (10–13 years) with ASD, age significantly moderated parasympathetic reactivity, with older youth demonstrating a particularly atypical reactivity pattern (Muscatello et al., 2021a, 2021b). In relation to psychopathologies, an examination of children 5–14 years with low- or high-risk of depression (based upon parent history of childhood-onset mood disorder) and without ASD demonstrated lower resting RSA in the high-risk group and a significant age by group effect, such that the

developmental slope of resting RSA was blunted in the high-risk group (Gentzler et al., 2012).

As mentioned, effects of biological sex on RSA are notable, which may have implications for the variability of findings regarding ANS function in ASD. In non-clinical samples, it has been reported that male youth have greater parasympathetic regulation relative to females (see Koenig et al., 2017 for review). Yet, a recent large-scale analysis found females reached peak PNS regulation at an earlier age than males, likely due to hormonal and pubertal timing differences between sexes (Harteveld et al., 2021). Studies of adult females show strong associations between hormonal changes associated with menstruation and the ovulatory cycle and heart rate variability (Schmalenberger et al., 2019, 2020; Tenan et al., 2014; Vallejo et al., 2005). Considering the prevalence of males and historical under-representation of females in autism research, studies of ANS regulation in ASD have often been restricted to all-male samples, with little or no investigation of sex differences in this population. One recent study of adolescents noted significant variability within a small sample of females with ASD (Edmiston et al., 2016); however, the sample was under-powered to further examine these sex differences and thus included only males in final analyses. A greater understanding of sex-based autonomic differences and their relationship to symptoms of ASD is critical, particularly during the pubertal period, where females with ASD may be particularly impacted by atypical development (Corbett et al., 2020).

To address remaining questions regarding the role of development and biological sex in ANS functioning of youth with ASD, the current study aimed to examine diagnostic differences in resting parasympathetic regulation over the course of 2 years in adolescents with ASD or TD. The study sought to provide a preliminary characterization of the extent to which pubertal development and biological sex further contributed to differences in PNS functioning in adolescents with ASD. It was hypothesized that youth with ASD would demonstrate lower resting-state RSA across the two timepoints (1 year apart) (Hypothesis 1). Pubertal development was predicted to be positively related with RSA, where advanced pubertal status and older age correlate with higher resting state PNS (Hypothesis 2). Finally, preliminary analyses explored between- and within-sex differences. Particular focus was placed on females with and without ASD as previous research by the study team (Edmiston et al., 2016) cited significant variability within a small sample of females with ASD.

## Materials and Methods

### Participants

Participants were enrolled in a large, longitudinal study of pubertal development in youth with and without ASD

(Corbett 2017). The current study includes data from Years 1 and 2 of the four-year study. At T1, 215 youth ages 10–13 years completed baseline RSA collection, including 123 youth with ASD (90 male, 33 female) and 92 TD (52 male, 40 female). Of these 215, a total of 184 participants (95 ASD, 89 TD) returned 1 year later for the T2 follow-up visits. Of those, 45 youth had incomplete data from an inability to complete in-lab visits, including RSA collection, due to the COVID-19 pandemic and associated shut-downs. There were no significant differences in age, IQ, T1 resting RSA, or BMI percentile between those who did and did not complete the full, in-person T2 study visit (all  $p > 0.05$ ).

An estimated 42% of children with ASD have been reported to take at least one psychotropic medication (Mire et al., 2014), thus the current study did not include a completely medication-naïve sample to be more representative of the overall ASD population. At T1, in the ASD group, 49.6% of youth were taking at least one psychotropic medication, while 9.8% of TD participants reported taking a daily psychotropic. At T2, distribution of medicated vs. non-medicated were similar, with 48.9% of ASD participants on psychotropics compared to 10.2% of TD participants.

To be included in the study, participants were required to have an intelligence quotient (IQ)  $\geq 70$  as estimated by the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II, Wechsler, 2011). Participants in the ASD group had a confirmed diagnosis according to Diagnostic and Statistical Manual-5 criteria (APA, 2013). Diagnoses were confirmed by: (1) a previous diagnosis by a psychologist, psychiatrist, or behavioral pediatrician with autism expertise; (2) current clinical judgment, and (3) corroborated by the Autism Diagnostic Observation Schedule (ADOS-2; Lord et al., 2012), a semi-structured interview-based instrument administered by research-reliable personnel. The TD group was defined as adolescents without ASD or other developmental delay and/or neurodevelopmental disorder based on parent screening. Parents completed the Social Communication Questionnaires (SCQ; Rutter et al., 2003), a parent-report questionnaire used to screen for ASD symptoms. To be included in the TD group, youth had to score  $< 10$  on the SCQ.

### Procedures

All study procedures were approved by the Vanderbilt University Institutional Review Board (IRB) and were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Parents/guardians and youth participants provided written consent and written and verbal assent, respectively.

Participation included two visits to the research laboratory at T1 and an additional visit 1 year later (T2). At the initial visit, all study procedures were discussed, and

consent/assent was obtained from the guardian and child, respectively. Following consent, eligibility was confirmed by administration of the ADOS-2 (Lord et al., 2012) for ASD participants and the WASI-2 (Wechsler, 2011) for all participants. Following confirmation of eligibility, youth underwent a brief physical examination and completed the resting-state RSA collection (procedures described below). Approximately 12 months after completion of T1, participants returned for the T2 visit, when physical examination and resting RSA collection were repeated.

### Pubertal Status

To obtain an objective measure of pubertal stage, participants completed a physical examination (PE), conducted by trained and licensed study physicians, to reliably identify pubertal development and determine Tanner stage (Marshall & Tanner, 1969, 1970). Tanner staging included two measures with 5 stages for Genitals (G1-G5 for boys) or Breasts (B1-B5 for girls; GB stage) and Pubic hair (P1-P5 for both genders; PH stage). Full procedures for the exam and reliability of the PE relative to parent- or self-reported measures have been previously described (Corbett et al. 2019). While GB staging is considered more reliable than PH staging (Emmanuel & Bokor, 2022), analyses were conducted for GB and PH scales separately for completeness and to be consistent with previous studies reporting on both staging categories (e.g., (Corbett et al., 2021b and Corbett et al., 2020)).

### Body Mass Index (BMI)

During the physical exam, height (in.) and weight (lb.) were collected by study physicians using a calibrated stand-on Health-o-meter TM Professional 499KL Waist High Digital Scale with Height Rod (Hogentogler & Co., MD, USA). BMI was calculated according to the standard formula ( $[\text{lb}/\text{in}^2] \times 703$ ) and converted into age- and sex-adjusted percentiles according to Center for Disease Control (CDC) growth charts for children and adolescents ages 2–19 years (<https://www.cdc.gov/healthyweight/bmi/calculator.html>).

### Resting Respiratory Sinus Arrhythmia (RSA)

RSA was collected using MindWare Mobile Impedance Cardiograph units (MindWare Technologies LTD, Gahanna, OH) for synchronized electrocardiography (ECG) and respiration data collection using a seven-electrode configuration. To reduce potential anxiety surrounding the protocol, electrodes were described as ‘stickers’, and visual guides were available to demonstrate location of the electrodes on the torso. If participants expressed significant discomfort from the electrode placement, the protocol was discontinued. Of

the 215 participants at T1, four participants with ASD were unable to complete the RSA collection procedures due to sensory sensitivities associated with the stickers. Additionally, 3 TD participants were missing baseline data due to equipment error or atypical heart rhythm. Data collection began following a 25-min acclimation period to the lab environment. Resting RSA was measured during a five-minute rest period in which all participants were instructed to sit quietly without engaging in any other tasks to reduce any potential confounding effects of movement or activity.

RSA was derived in accordance with guidelines set forth by the Society for Psychophysiological Research committee on heart rate variability (Task Force, 1996). ECG signal was sampled at 500 Hz and analyzed using the Heart Rate Variability Software Suite provided by MindWare Technologies (MindWare Technologies LTD, Gahanna, OH). RSA was quantified as the natural logarithm of integral power within the respiratory frequency (high frequency) band (0.12 to 0.42 Hz), and respiration was monitored by impedance cardiography. The respiration signal was displayed to ensure that values were within the designated frequency band. Respiratory frequency was confirmed to lie within the high frequency/RSA band (0.12–0.42 Hz) for all participants. Of the total processed data, 3.5% were excluded due to excessive motion artifact, equipment error, or cardiac arrhythmias. RSA was measured as  $\ln \text{ms}^2$ .

### Statistical Analysis

Demographic variables were compared between ASD and TD groups using independent sample *t*-tests. If the assumption of equal variance was violated, the Welch degree of freedom approximation was used. RSA values were normally distributed and free of extreme outliers.

Hypotheses were tested using linear mixed effects models with a random intercept for subject to account for correlation within subjects. To test the hypothesis that resting RSA across T1 and T2 differed by diagnosis and sex, we fit a model of resting RSA with main effects for sex, diagnosis, and time. Psychotropic medication use was included as a covariate in all models. To test the extent to which change in RSA levels over time differed by diagnostic status, an interaction term between diagnosis and time was included. To examine hypothesized relationships between RSA and physical development, an additional model was fit including age, GB stage, and BMI as potential covariates or effect modifiers. The model was repeated using PH stage as well. Preliminary analyses were carried out in males and females separately to observe potential differences in resting RSA and development within-sex. Robust effect size index (*S*) (Vandekar et al., 2020) was computed, where *S* is equal to  $\frac{1}{2}$  Cohen’s *d*. Thresholds were interpreted according to Cohen (Cohen, 1988) for small ( $d=0.2$ ,  $S=0.1$ ), medium ( $d=0.5$ ,

$S = 0.25$ ) and large ( $d = 0.8$ ,  $S = 0.4$ ) effects. All analyses were performed using IBM SPSS Statistics 28 (“IBM SPSS Statistics for Mac,” 2021).

## Results

Demographic and diagnostic information is presented in Table 1. Groups did not differ on age,  $p > 0.05$ . While there was a significant difference between groups based upon IQ,  $t(211.88) = 7.07$ ,  $p < 0.001$ , the ASD group fell well within the average range. As expected, ASD youth had significantly higher SCQ scores,  $t(150.98) = -18.42$ ,  $p < 0.001$ , and, consistent with previous reports (Corbett et al., 2021), average BMI percentile was significantly elevated in the ASD group,  $t(211) = -2.61$ ,  $p = 0.01$ .

## Diagnosis and Developmental Effects on RSA

To test Hypothesis 1 anticipating diagnostic differences in resting RSA, we fit a model with main effects for sex, diagnosis, time, and an interaction term for diagnosis and time. To account for diagnostic differences in psychotropic medication use, this was included as a covariate of interest in the model. Results are presented in Table 2, and parameter estimates are available in Supplemental Table 1. As shown in Fig. 1, resting state RSA significantly increased from T1 to T2,  $p = 0.034$ , particularly within the TD group. There was a significant main effect for diagnosis, with lower RSA in youth with ASD,  $p = 0.046$ , as well as an interaction between diagnosis and time,  $p = 0.05$ , demonstrating a small effect size,  $S = 0.11$ . Specifically, the ASD group had a blunted change in RSA from T1 to T2 (see Fig. 1). Notably, psychotropic medication use was a significant covariate of interest,

**Table 1** Descriptive statistics at time 1

	N	TD		ASD	
		(n = 92)		(n = 123)	
		M	SD	M	SD
Age	215	11.64	1.18	11.38	1.04
Full scale IQ*	215	117.82	13.82	101.53	19.92
ADOS total	123	–	–	12.32	4.34
SCQ*	214	2.71	2.52	17.35	8.31
Percentile BMI*	213	54.43	31.79	65.67	30.63
	N	TD Proportion		ASD Proportion	
Sex: female*	215	0.43 40/92		0.27 33/123	
Race	215				
Caucasian		0.88 81/92		0.82 101/123	
African American*		0.02 2/92		0.11 14/123	
American Indian		0.00 0/92		0.00 0/123	
Asian or Pacific Islander		0.00 0/92		0.01 1/123	
More than one		0.10 9/92		0.06 7/123	
GB stage	212				
Stage 1		0.41 37/90		0.42 51/122	
Stage 2		0.33 30/90		0.29 35/122	
Stage 3		0.17 15/90		0.14 17/122	
Stage 4		0.09 8/90		0.13 16/122	
Stage 5		0.00 0/90		0.02 3/122	
PH stage	212				
Stage 1		0.52 46/89		0.54 66/123	
Stage 2		0.18 16/89		0.12 15/123	
Stage 3		0.18 16/89		0.19 23/123	
Stage 4		0.10 9/89		0.13 16/123	
Stage 5		0.02 2/89		0.02 3/123	

TD typically developing, ASD autism spectrum disorder, M mean, SD standard deviation, IQ intelligence quotient, BMI body mass index, GB genital/breast stage, PH pubic hair stage

\*Indicates significant difference ( $p < 0.05$ ) between groups

$p=0.02$ , where taking psychotropics was associated with lower RSA. There was no significant main effect for sex,  $p>0.05$ .

Hypothesis 2 predicted positive associations with advanced physical development. Thus, a second model was fit with BMI, GB stage, and age. As shown in Table 3, age and GB stage did not contribute substantially over the effects of other covariates,  $p>0.05$ . Therefore, we did not find sufficient evidence for an association between age and GB stage with RSA values. Consistent with previous reports (Muscatello et al., 2021a), there was a significant main effect for BMI,  $p=0.017$ , with elevated BMI associated with lower

resting RSA (see Supplemental Table 2 for parameter estimates). The main effect for diagnosis was no longer significant,  $p>0.05$ , while psychotropic medication use remained a statistically significant predictor of RSA,  $p=0.005$  with a trend diagnosis and time interaction with small effect,  $p=0.06$ ,  $S=0.12$ .

The model was repeated, including PH stage instead of GB stage. Similarly, PH stage was not associated with RSA,  $p>0.05$  (Supplemental Table 3).

### Preliminary Within-Sex Analysis

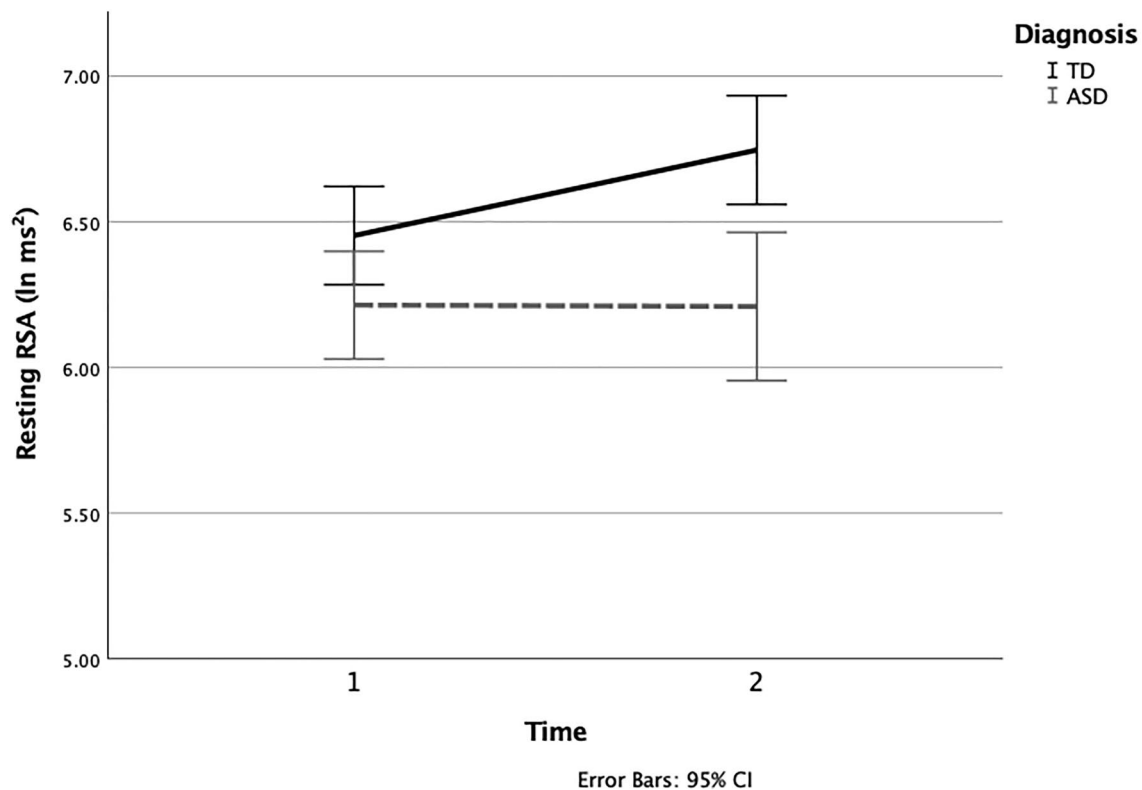
As reported above, there were no significant between-sex differences in the primary models. However, the unequal distribution of males and females, with a notable male bias, may have left models underpowered to detect sex-based differences. To explore differences within males and females with or without ASD, preliminary analyses were conducted separately for males and for females. In males, there was neither a significant effect for diagnosis,  $p=0.72$ ,  $S=0.0$ , nor for the diagnosis and time interaction,  $p=0.11$ ,  $S=0.11$  (See Table 4; Fig. 2). However, an increase in GB stage predicted an increase in RSA,  $p=0.025$ ,  $S=0.18$ , while medications,  $p=0.025$ ,  $S=0.18$ , and BMI,  $p=0.01$ ,  $S=0.21$ , predicted lower RSA in males.

**Table 2** Type 3 sum of squares ANOVA table to test main effects and interaction for diagnosis and time

Factor	<i>F</i>	<i>Df</i>	<i>p</i>	Effect size ( <i>S</i> )
Sex	0.06	1, 208.19	0.81	0.00
Diagnosis	4.01	1, 225.03	<b>0.05</b>	0.12
Time	4.59	1, 152.68	<b>0.03</b>	0.13
Medication	5.84	1, 259.35	<b>0.02</b>	0.15
Diagnosis*time	3.88	1, 153.74	<b>0.05</b>	0.11

Significant *p*-values in bold

*Df* degrees of freedom



**Fig. 1** Resting RSA from Time 1 to Time 2 by Diagnosis. Youth with ASD demonstrated significantly lower resting RSA across Times 1 and 2 and a blunted developmental change (slope) in RSA

**Table 3** Type 3 sum of squares ANOVA table of main effects including age, BMI, and GB stage

Factor	<i>F</i>	<i>Df</i>	<i>p</i>	Effect size ( <i>S</i> )
Sex	0.36	1, 204.11	0.55	0.00
Diagnosis	1.37	1, 224.97	0.24	0.04
Time	1.02	1, 299.48	0.31	0.01
Age	0.15	1, 239.93	0.70	0.00
Percentile BMI	5.81	1, 252.76	<b>0.02</b>	0.15
GB stage	1.61	1, 326.02	0.20	0.05
Medication	8.03	1, 251.01	<b>0.005</b>	0.19
Diagnosis*time	3.71	1, 153.06	0.06	0.12

Significant *p*-values in bold*Df* degrees of freedom**Table 4** Type 3 sum of squares ANOVA table of main effects within males or females

Factor	<i>F</i>	<i>Df</i>	<i>p</i>	Effect size ( <i>S</i> )
<b>Males</b>				
Diagnosis	0.13	1, 145.49	0.72	0.00
Time	0.69	1, 209.39	0.41	0.00
Age	0.33	1, 169.20	0.57	0.00
Percentile BMI	6.82	1, 169.99	<b>0.01</b>	0.21
GB Stage	5.12	1, 223.45	<b>0.02</b>	0.18
Medications	5.11	1, 172.96	<b>0.02</b>	0.18
Diagnosis*time	2.61	1, 99.93	0.11	0.11
<b>Females</b>				
Diagnosis	1.65	1, 69.61	0.20	0.10
Time	1.09	1, 73.39	0.30	0.04
Age	2.89	1, 68.44	0.09	0.17
Percentile BMI	0.08	1, 83.41	0.78	0.00
GB Stage	3.42	1, 93.29	0.07	0.20
Medication	7.44	1, 72.16	<b>0.01</b>	0.32
Diagnosis*time	2.60	1, 52.90	0.11	0.16

Significant *p*-values in bold*Df* degrees of freedom

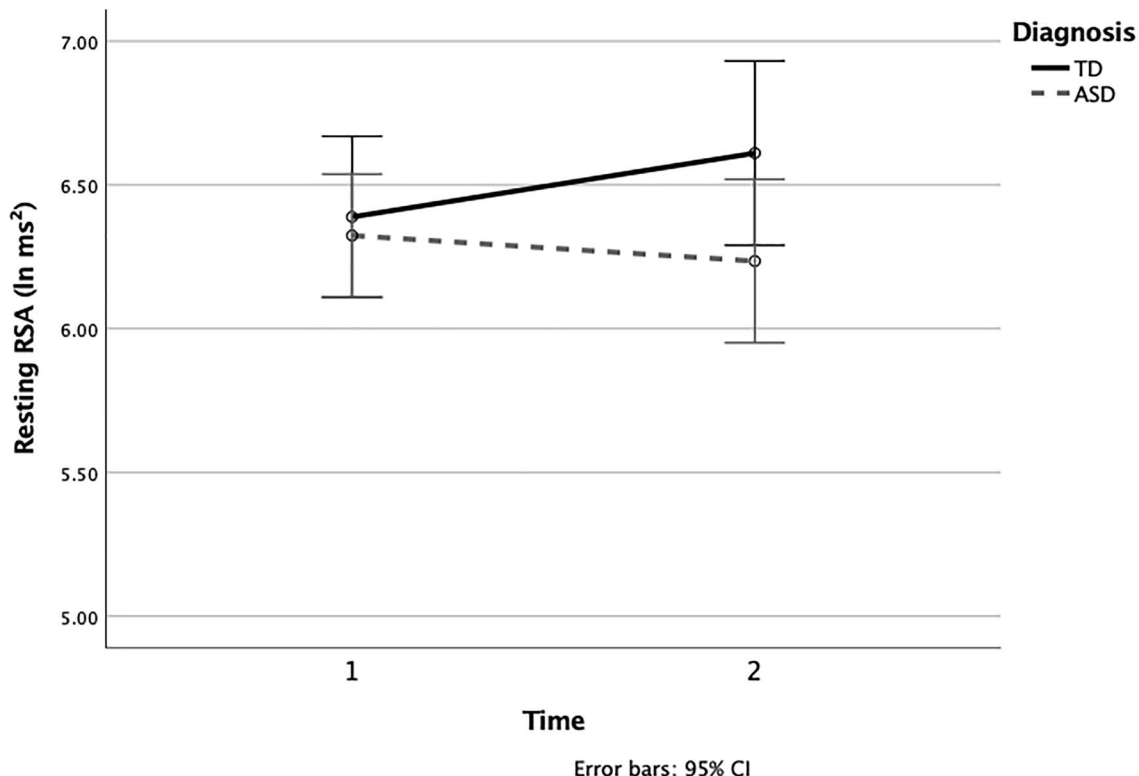
A different pattern of effects was observed in females. While diagnosis was similarly not statistically significant, it did maintain a small effect,  $S = 0.10$  (Table 4). As shown in Fig. 3, females with ASD tended to have lower resting state RSA. Further, the diagnosis and time interaction did not reach the level of statistical significance,  $p > 0.05$ , but showed a small effect,  $S = 0.16$ . In contrast to males, BMI was not a significant predictor of RSA,  $p > 0.05$ . Notably, psychotropic medication use was a particularly strong predictor of lower RSA in females,  $p = 0.01$ ,  $S = 0.32$ . Parameter estimates are presented in Supplemental Tables 4 and 5.

## Discussion

To our knowledge, this study is the first to longitudinally examine resting state parasympathetic function in adolescents with ASD. Findings demonstrated youth with ASD have consistently lower resting RSA relative to their TD peers. Notably, the expected developmental increase in resting RSA may be slowed in ASD. Pilot analyses within males and females further suggest a unique phenotype characterized by lower RSA may be especially prevalent in females with ASD, particularly those taking psychotropic medications. The apparent developmental differences in PNS regulation over two timepoints, separated by 1 year, provide preliminary evidence for persistent autonomic dysfunction in ASD, which may worsen relative to TD peers throughout the adolescent period.

As predicted by Hypothesis 1, RSA in youth with ASD was significantly lower compared to youth with TD. Findings contribute to a growing literature citing reduced PNS functioning across the lifespan (e.g., Patriquin et al., 2019). This diminished parasympathetic regulation has been associated with more severe social symptoms in ASD (Edmiston et al., 2016; Neuhaus et al., 2014; Patriquin et al., 2013; Vaughan Van Hecke et al., 2009). Further, research in non-ASD populations cites atypical ANS regulation in psychopathological conditions, including, anxiety (e.g., Chalmers et al., 2014; Thayer et al., 1996), depression (e.g., Koenig et al., 2016; Rottenberg et al., 2007), and Post-Traumatic Stress Disorder (e.g., Campbell et al., 2019). Given the prevalence of both physiological dysfunction and psychological comorbidities, ongoing efforts have sought to determine whether the two are related in ASD. Some findings have reported correlations between autonomic regulation and response with internalizing symptoms (e.g., anxiety, depression) in ASD (Kushki et al., 2013; Muscatello et al., 2020; Neuhaus et al., 2014; Panju et al., 2015), while other studies in ASD have been unable to demonstrate a link between autonomic regulation and anxiety or depression (Hollocks et al., 2014, 2016; Kushki et al., 2014). Considering the current findings of lower RSA in ASD throughout the early adolescent years, it will be important to examine the trajectory of autonomic functioning as a potential marker of risk for, or resilience to, later internalizing diagnoses.

In the current study, a diagnosis and time interaction was observed, in which youth with ASD had a blunted slope in RSA change from T1 to T2. Research suggests that normative development of the PNS includes a gradual increase in regulation throughout childhood, with a plateauing around late-childhood and adolescence (e.g., Gatzke-Kopp & Ram, 2018; Hartevelde et al., 2021; Hin-nant et al., 2011). Moreover, at least one study in typically



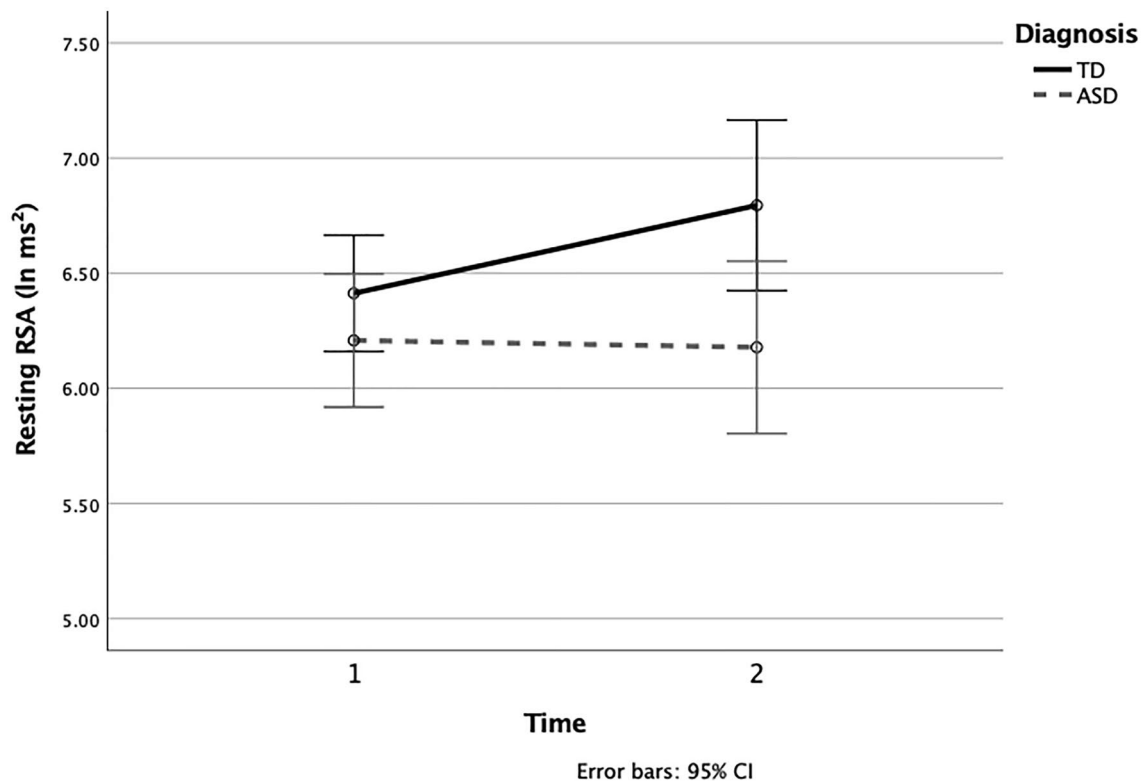
**Fig. 2** Resting RSA from Time 1 to Time 2 in Males with ASD and TD. Plot depicts model effects of diagnosis on RSA, averaged across age, GB stage, and BMI percentile. No diagnostic difference was observed in males with and without ASD at Times 1 or 2

developing individuals demonstrated differential parasympathetic reactivity to stress in younger versus older youth (El-Sheikh, 2005). Cross-sectional analyses of physiological systems support age and developmental differences in ASD. For example, studies of school-aged youth found evidence of decreasing heart rate in older youth both with and without ASD (Harder et al., 2016; Kushki et al., 2014). However, there were no age effects in cardiac autonomic function, particularly parasympathetic-mediated RSA (Harder et al., 2016). In stress reactivity, recent evidence reported that youth with ASD had lower RSA response to social interaction relative to same-aged TD peers, and with diagnostic differences especially prevalent in the older ASD youth (Muscatello et al., 2021a, 2021b). Furthermore, examination of related stress systems, such as the hypothalamic–pituitary–adrenal axis, have reported diagnostic differences in physiological functioning to be positively associated with older age in ASD children and adolescents (e.g., Corbett et al., 2010, 2021a, 2021b; Muscatello & Corbett, 2018; Schupp et al., 2013). The current study suggests a delayed developmental slope may be associated with an autism diagnosis, emphasizing the need for future research into whether atypical developmental trajectories persist throughout later stages of puberty and

are associated with physical, behavioral, and/or psychological outcomes.

Despite the within-individual change in RSA over the two timepoints, neither pubertal development (Tanner stage) nor age was significantly associated with average resting RSA in the full sample. However, genital/breast stage was associated with elevated RSA in males and was at trend in females. It may be that the differences in timing and tempo between males and females (Biro et al., 2001; Marceau et al., 2011; Mendle et al., 2010) are important for considering developmental influence on physiology, such as RSA, particularly in ASD (Corbett et al., 2020). It should also be acknowledged that developmental change in ANS functioning is complex, as a number of contextual factors (e.g., family stress, poverty) may contribute to individual differences and variability (Gatzke-Kopp & Ram, 2018; Hinnant et al., 2011). Furthermore, despite the longitudinal nature of the study, the total age range of the current study sample remained relatively narrow at ages 10–14 years. During the 1 year especially, the majority of participants were at the earlier stages of pubertal development (Tanner 1 and 2). The current sample will be followed longitudinally for an additional 2 years, and it is expected that pubertal and age effects will become evident as the participants progress into mid- to late-adolescence. A





**Fig. 3** Resting RSA from Time 1 to Time 2 in Females with ASD and TD. Plot depicts model effects of diagnosis on RSA, averaged across age, GB stage, and BMI percentile. Females with ASD showed lower

RSA compared to TD females, though this did not reach the level of statistical significance ( $p < .05$ )

more intensive investigation of pubertal timing and tempo in ASD from early to late puberty, considering other contextual factors such as sex, race, and trauma, among others, will more completely outline the extent to which development of the parasympathetic system is related to the broader autism phenotype.

Lastly, the current study sought to examine between- and within-sex differences in autonomic function, particularly in ASD. Primary models were not significant for a main effect of sex (male vs. female). However, the distribution of males versus females was heavily biased towards males, likely hindering power for detecting differences in females. As such, preliminary analyses were carried out separately for males and for females to examine diagnostic effects within sex. As reported and displayed in Figs. 2 and 3, the diagnostic effect for lower RSA in ASD was more pronounced in females, though neither group reached the level of statistical significance. Nevertheless, this finding may be consistent with previous research noting significant variability in RSA in female adolescents with ASD (Edmiston et al., 2016).

Evidence in typically developing youth and adults supports sex-based differences in autonomic functioning. For example, females tend to demonstrate reduced vagal activity and increased heart rate compared to their male peers

(Koenig et al., 2017). Hormonal influences are particularly notable for females, with reported differences in HRV according to the phase of the menstrual cycle (Schmalenberger et al., 2019, 2020). As Koenig and Thayer (2016) explain, lower vagal activity in females, particularly during the adolescent period, may have significant clinical implications. Namely, reduced PNS activity is associated with psychopathologies, including depression (e.g., Koenig et al., 2016), with higher prevalence rates in females relative to males (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993). Concerningly, an estimated 40% of adults with ASD will be diagnosed with a depressive disorder in their lifetime (Hollocks et al., 2019; Hudson et al., 2019), well-above the global population average (Mayes et al., 2011). The combination of increased prevalence based upon female sex and an autism diagnosis suggests an additive effect, which leaves females with ASD particularly susceptible (Schwartzman et al., 2022). As such, a close examination of sex differences in parasympathetic regulation and implications for later development of internalizing conditions may be especially critical in female adolescents with ASD.

Lastly, the contribution of psychotropic medication use cannot be ignored. As previously described, reductions in RSA have been frequently cited within several psychiatric

diagnoses, such as anxiety and depression (Koenig et al., 2016; Thayer et al., 1996). It has been hypothesized that use of psychotropic medication, particularly antidepressants, moderates the observed relationship between psychiatric disorders and reduced parasympathetic regulation (Licht et al., 2010). However, a recent large meta-analysis of 170 studies concluded that medications had only a small impact on autonomic function in otherwise healthy participants with a psychiatric disorder (Alvares et al., 2016). In our study, we did find psychotropic medication use to be predictive of RSA. Considering the large number of youth with ASD reported to be taking psychotropics (Mire et al., 2014), it will be important for future studies to closely examine the extent to which medication use and other health factors contribute to the lower RSA reported across numerous studies of ASD youth.

## Limitations

The current study was strengthened by the longitudinal examination of resting RSA to examine changes in PNS function over 2 years. Pubertal development was measured using a gold-standard physician-conducted physical examination. Despite these strengths, limitations do exist. First, the sample was relatively limited to early adolescence, whereas important differences may emerge later in development. Future studies following the current sample over a total of 4 years will ultimately provide a more nuanced overview of parasympathetic development in ASD throughout a broader range of puberty and adolescence. Relatedly, the two assessments were one-year apart, and it remains unclear the extent to which group differences may change, if at all, over the course of several years. Secondly, the total number of females in the current sample limited our ability to closely examine sex differences within diagnoses. Preliminary findings noted lower PNS regulation in ASD, particularly ASD females. Thus, future studies in a much larger sample of females will provide insight into the effects of sex on PNS development. Additionally, the sample was limited to individuals with an IQ > 70 and who predominantly identified as White. While the sample included youth on medications to be more inclusive and representative of the population, the current study did not examine the possible effects of different types of medications on physiological functioning. Future efforts may aim to include more diverse samples with a broad range of cognitive functioning and from underrepresented groups to expand conclusions across the full range of the autism spectrum and account for potential RSA development differences based upon IQ or race. It will also be important to further consider the role of possible confounding variables, including medication use, BMI changes, or occurrence of mood and other comorbid conditions in

expanded longitudinal studies with a diverse sample. In addition to attrition, a common challenge in longitudinal studies, the T2 sample was affected by the early months of the COVID-19 pandemic and associated shutdowns. We were unable to collect second timepoint RSA data for several participants as all research visits were moved to a virtual format and limited to psychological testing and behavioral reports. Finally, the current study relied on a sole measure of parasympathetic function, namely, RSA; however, some research suggests RSA may not be a perfect metric of pure cardiac vagal tone (Grossman & Taylor, 2007). Future studies will be pursued in which multiple autonomic measures are included, such as those indexing both parasympathetic and sympathetic function, to provide a more comprehensive understanding of autonomic function or dysfunction in ASD. Nevertheless, the current study provides critical preliminary evidence for an extended difference in parasympathetic influence within ASD, which warrants further exploration.

## Conclusion

The current study provides preliminary support for atypical developmental trajectories of parasympathetic regulation in ASD. Over a two-timepoint period (Years 1 and 2 of a longitudinal study), adolescents with ASD demonstrated lower RSA, with differences in the 2nd year exacerbated by an apparent blunted trajectory in ASD. While no direct evidence was observed for either pubertal stage or age as a predictor of resting RSA across the full sample, participants will be followed for a total of 4 years, so that relationships across the broader developmental range may be revealed. Finally, preliminary evidence of within-sex differences suggest diagnostic effects may be especially apparent for females with ASD. It will be necessary to consider potential clinical implications of prolonged ANS dysfunction, and future studies will continue to elucidate the impact of physiological functioning on sociobehavioral outcomes as well as co-occurring psychopathological conditions in individuals with ASD.

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**Author Contributions** RAM contributed to the design of the study, led data acquisition, analyzed physiological data, analyzed and interpreted the data, and wrote the initial draft of the manuscript. AP contributed to data acquisition, scheduling of protocols, physiological data analysis, and editing of the final manuscript. AR and IS contributed to collection and analysis of physiological data and editing of the final manuscript. BAC designed the study, oversaw data acquisition, analysis, and interpretation, and contributed to writing the initial draft of the manuscript. All authors participated in the preparation and editing of the manuscript and approved the final manuscript.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical stands of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent** Informed written consent and assent was obtained from all parents and study participants, respectively, prior to inclusion in the study.

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