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Review

Anxiety and physiological responses to the Trier Social Stress Test for Children in adolescents with cyclic vomiting syndrome

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ABSTRACT

This study compared anxiety and physiological responses during the Trier Social Stress Test for Children (TSST-C) in adolescents. 38 subjects (26 females) were enrolled: 11 cyclic vomiting syndrome (CVS), 11 anxiety, and 16 controls. Salivary cortisol, α-amylase and heart rate variability (HRV) were assessed during the TSST-C. Anxiety was measured by the Screen for Childhood Anxiety Related Emotional Disorders (SCARED), Anxiety Disorders Interview Schedule, and State-Trait Anxiety Inventory for Children (STAI-C). 11 anxiety and 7 CVS subjects had ≥1 anxiety disorder. 82% in the anxiety and CVS groups met criteria for an anxiety disorder on the SCARED. Combining groups, cortisol increased from baseline to recovery during the TSST-C ($p = 0.0004$) and the stressor to recovery ($p = 0.005$). α-amylase did not differ during the TSST-C for the total sample, but increased for anxiety compared to controls from baseline to recovery ($p = 0.01$). HRV decreased during the stressor ($p = 0.0001$) and increased at recovery ($p = 0.004$). No associations were found between biomarkers and trait anxiety. Associations were found between baseline HRV and pre-test state anxiety ($r = -0.406$, $p = 0.012$) and between recovery HRV and post-test state anxiety ($r = -0.501$, $p = 0.002$) for the total sample. Anxiety is prevalent in CVS warranting screening. HRV may serve as a biomarker for evaluating stress as a potential trigger for CVS episodes. State but not trait anxiety was associated with changes in HRV, suggesting acute anxiety may be more relevant in linking stress and CVS episodes.

Contents

Abbreviations: CVS, cyclic vomiting syndrome; EKG, electrocardiogram; HPA, hypothalamic-pituitary-adrenal; HRV, heart rate variability; ICC, intraclass correlation coefficient; RMSSD, root-mean square differences of successive R-R intervals; SCARED, Screen for Childhood Anxiety Related Emotional Disorders; STAI-C, State-Trait Anxiety Inventory for Children; TSST-C, Trier Social Stress Test for Children.

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1. Introduction

Cyclic vomiting syndrome (CVS) is characterized by stereotypic episodes of intense nausea and vomiting, with normal health between episodes. The mechanisms of CVS are not well understood. Several population-based studies estimate the prevalence of pediatric CVS to be approximately 2% (Abu-Arafeh and Russell, 1995; Ertekin et al., 2006). For most youth (82%), CVS is thought to be a migraine variant based on similarities in presentation (i.e., on-off pattern, nausea, abdominal pain, vomiting), response to anti-migraine therapies and family history of migraines (Li et al., 1999; Stickler, 2005; Tarantino et al., 2014). As with migraine, stress is often cited as a trigger for CVS episodes (Li et al., 2008), but it is unclear how the child's stress exposure precipitates a CVS episode.

Youth with CVS have also been found to have a high incidence of anxiety and mood disorders that may impact their ability to cope with stress (Drumm et al., 2012; Forbes et al., 1999; Tarbell and Li, 2008). Children with anxiety disorders are reported to have altered hypothalamic-pituitary-adrenal (HPA) axis and autonomic functioning resembling chronic stress (Dieleman et al., 2015). There is also evidence of altered autonomic function in youth with anxiety disorders (Boyce et al., 2001; Sharma et al., 2011b) and in those with CVS (Chelimsky and Chelimsky, 2007; To et al., 1999).

While clinical reports have shown that stress can trigger a CVS episode, measurement of specific physiological and psychological responses to stress in subjects with CVS in a controlled experimental paradigm has not been studied. The aim of this pilot study was to compare anxiety, changes in hypothalamic-pituitary-adrenal axis (HPA) including salivary cortisol and α-amylase, and autonomic responses during a standardized stress challenge in 3 pediatric groups: subjects with CVS, an anxiety disorder, and healthy controls.

2. Materials and methods

2.1. Participants

Subjects (ages 13–18 years) and their parents were recruited and divided among 3 groups: 1) those who met the international consensus criteria for CVS (Li et al., 2008) were the experimental subjects, 2) those with a diagnosed anxiety disorder (e.g., social phobia, generalized anxiety and/or separation anxiety disorder) as the comparison group, and 3) healthy youth with no major medical or psychiatric illnesses as the controls. We excluded children who were not English speaking or who had other major medical or developmental disorders or psychiatric comorbidity other than anxiety or mood disorders. Participants were recruited from pediatric gastroenterology and pediatric psychiatry clinics, as well as blast emails sent to a university community, an internet posting on the Cyclic Vomiting Syndrome Association website, and flyers placed in community settings. Interested youth and parents were screened for study eligibility and then scheduled for a clinic visit to obtain informed consent/assent and undergo experimental procedures. Participants received a $50 stipend for participation. The Institutional Review Board approved this study.

2.2. Measurements

2.2.1. Demographic and medical information

These data were collected during parent or youth interviews and a review of the electronic medical record for participants that were recruited from the hospital's clinical services. Medical record review was used to confirm diagnoses for participants in the CVS and the anxiety groups. Frequency and duration of CVS episodes were obtained for youth with CVS.

2.2.2. Diagnosis of cyclic vomiting syndrome

The diagnostic criteria included: 1) recurrent severe, discrete episodes of vomiting; 2) normal health between episodes; 3) duration of vomiting from hours to days; and 4) no apparent cause of vomiting, as well as supportive criteria that the episodes be stereotypical and self-limited (Li et al., 2008).

2.2.3. Psychiatric diagnoses and psychiatric symptom measures

The child and parent versions of the Anxiety Disorders Interview Schedule for Children (ADIS-C) (Silverman and Albano, 2004) based on the DSM-IV, were used to evaluate all adolescent participants for the presence of anxiety or depressive disorders. Two clinical psychologists conducted the interviews. A subset of the ADIS-C interviews was audiotaped to review for fidelity of administration. Anxiety symptoms were assessed with the Screen for Childhood Anxiety Related Emotional Disorders (SCARED) (Birmaher et al., 1999). This 41-item questionnaire, validated for use in children 8 years of age and older, is based upon the DSM-IV criteria for anxiety disorders in children. This scale screens for symptoms associated with specific anxiety disorders as well as behaviors such as school avoidance that are common across anxiety disorders in children. Total SCARED scores may range from 0 to 82, with scores 25 or greater indicative of clinically significant anxiety symptoms. Both the child self-report and parent proxy versions of the SCARED were used. The total SCARED scores were used to compare the study groups.

2.2.4. Standardized stress test

The Trier Social Stress Test for Children (TSST-C) (Buske-Kirschbaum et al., 1997) which includes a social stressor (telling a story in front of two neutral judges after 5 min of preparation time) and a cognitive stressor (mental arithmetic-counting backward from 1081 by 7 s) was used to elicit psychological and physiological stress responses in the participants. These two stressors in combination are designed as a

motivated performance task that includes elements of uncontrollability and high levels of social-evaluative threat to produce the largest HPA axis stress responses (Dickerson and Kemeny, 2004). The TSST-C was administered in accordance with the method detailed by Buske-Kirschbaum et al. (Buske-Kirschbaum et al., 1997), with the exception that the test was administered with the participant sitting to control for orthostatic influences on physiological responses. The youth underwent physiological monitoring and provided self-reports of acute anxiety before and after the TSST-C.

2.2.5. Acute anxiety

The State-Trait Anxiety Inventory for Children (STAI-C) (Speilberger et al., 1973), state version, was used to assess acute anxiety before and after the administration of the TSST-C. This questionnaire consists of 20 words describing current emotions (e.g., nervous, upset) with intensity rated on a scale of 1–3. The total score was used to compare acute anxiety before and after the TSST-C.

2.2.6. Physiological measures

Measures of HPA axis activation included salivary cortisol and α-amylase obtained during the TSST-C. Salivary cortisol was used as it has been shown to increase in response to acute stressors (Dorn et al., 2003; Gaab et al., 2003; Sgoifo et al., 2003). Salivary α-amylase was also measured as it correlates with and is a surrogate for plasma catecholamine levels, particularly norepinephrine, under a variety of stressful conditions (Chatterton et al., 1996). Saliva was collected with the SalivaBio collection aid and tubes, kept cold, and stored at −15 °C until assayed. Trident regular flavor gum was used to help with saliva production if needed.

Heart rate variability (HRV) was used to assess autonomic response to the TSST-C. Electrocardiogram (EKG) and respiratory sensors acquired data (rate of collection: 256 samples per second) for calculation of HRV using the Biotrace + Software loaded on a laptop computer, interfaced wirelessly with the Nexus-10 encoder (Mind Media B.V., Netherlands) to record the physiological data. Heart rate signals from the time domain (inter-beat intervals), the root-mean square differences of successive R-R intervals (RMSSD), was used to calculate HRV. Both software generated artifact identification and visual inspection were used to eliminate artifacts in the inter-beat intervals used to calculate HRV.

2.3. Experimental protocol

Study visits were scheduled between 3:00–5:00 p.m. to control for diurnal variation in cortisol. Youth were asked to avoid eating and taking medications 1 h before testing due to the potential of food to alter salivary cortisol. The TSST-C was performed during an asymptomatic inter-episode period in the CVS patients.

Ten minutes prior to the administration of the TSST-C, baseline monitoring began.

The first 5 min of the physiological monitoring were used as a period for the participant to become accustomed to the recording devices, with the next 5 min serving as the baseline for measures of RMSSD, salivary cortisol and α-amylase. Participants completed the State version STAI-C. Saliva samples were obtained 10 min and 1 min before the TSST-C, between the public speaking and mental arithmetic components of the TSST-C, and 1 and 10 min after the TSST-C, time intervals that have been used in prior research (Buske-Kirschbaum et al., 1997; Dorn et al., 2003). The TSST-C itself took approximately 15 min. HRV was averaged over 5 min intervals including the baseline, the social stress and the mental arithmetic tasks, and a 10-min recovery period after the TSST-C. The STAI-C was repeated upon completion of the TSST-C.

2.3.1. Laboratory analyses

Cortisol concentration in saliva was determined using a commercial expanded range high sensitivity enzyme immunoassay kit (No. 1-3002/1-3012, Salimetrics, range 0.003–3.0 mg/dL). Standard curves were fit using commercial software (Revelation 3.2) for the ELISA plate reader (Dynex MRX) and unknowns computed. Laboratory controls included on every plate for determination of inter- and intra-assay coefficients of variability are <6% respectively for cortisol. Salivary α-amylase was determined using a commercial assay (1-1902, Salimetrics, range 3.1–423.1 U/mL). Briefly a substrate was added to diluted saliva to produce a chromogen in proportion to enzyme present. Intra and inter-assay coefficients of variation are <8% for high and low laboratory controls.

2.4. Statistical analyses

Descriptive data analyses were performed with SPSS Statistics for the Macintosh, Version 23 (IBM, Armonk, New York). Cronbach's α was used to evaluate the internal reliability of the STAI-C and the SCARED in the study sample. Intraclass correlation coefficients (ICCs) were used to evaluate agreement between parent and child reports of anxiety symptoms on the SCARED. ICC values were interpreted as 0.40, poor agreement; 0.41–0.60, moderate agreement; 0.61–0.80 good agreement; and 0.81 and higher, excellent agreement. Rates of psychiatric disorders among the 3 groups were compared using Chi Square tests. One-way ANOVA was used to evaluate group differences in state anxiety before and after the TSST-C. Paired *t*-tests were used to evaluate changes in state anxiety prior to and after the TSST-C within subjects. Effect sizes were calculated with Cohen's *d*, with 0.20 interpreted as a small effect, 0.60 a medium effect, and 0.80 and as a large effect. Correlation coefficients were used to examine associations between child reported state anxiety and RMSSD. Missing data for any dependent variable were excluded from analyses.

Spaghetti plots of cortisol, amylase and RMSSD were generated. Linear mixed models were used to determine whether a difference existed between the TSST-C intervals for the primary outcomes and, if that difference differed between the study groups. The role of anxiety as a potential confounder (child reported SCARED total score) was explored using a one-way ANOVA and linear mixed models. The role of medication use as a potential confounder was explored using Fisher's exact test and linear mixed models. Variables were log transformed as necessary. R version 3.1.1 software (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/) was utilized.

3. Results

3.1. Demographic data

We enrolled 38 subjects; 16 in the control group, 11 in the cyclic vomiting group and 11 in the anxiety group over 4 years. This was below our planned recruitment of 15 participants in each group, and thus our power to detect differences led us to combine the groups for a portion of the analyses described below. The mean age of the sample was 15.2 years (SD = 1.6). A majority of the participants were female (n = 26, 68%). Minority youth comprised 26% (n = 10) of the sample. There were no significant differences between the groups with regard to age, gender, ethnicity or body mass index. Average annual frequency of CVS episodes for youth with CVS was 22 (SD = 15) with episode duration ranging from 3 to 7 days. Three of CVS subjects had mild intermittent nausea and vomiting (1–3 emeses) between CVS episodes.

3.1.1. Medications

One youth in the control group was on a stimulant medication. Seven youth in the Anxiety group were on medications for management of their psychiatric symptoms, including: SSRIs (n = 6) and other psychiatric medications (n = 6). Four youth in the CVS group were on medications for management of their CVS: antiemetic (n = 2), tricyclic

antidepressant (n = 2), triptan (n = 1), or their anxiety SSRI (n = 3). There was a significant difference in the proportion of subjects on SSRI and/or TCA medications among the three groups; p = 0.0004, with no subjects in the control group on these medications. There was not, however, a significant association between these medications and the measured biomarkers.

3.2. Psychiatric assessments

3.2.1. Structured psychiatric diagnostic interviews

Thirty-five parents and 38 youth completed the ADIS-C. Ten sets of interviews (26%) were audiotaped and reviewed by a clinical psychologist for fidelity of administration. No administration variances were identified. Diagnoses by participant group are presented in Table 1. There was a significant difference among the participant groups with regard to the presence of anxiety disorders by child (Chi Square = 19.24, p ≤ 0.0001) and parent report (Chi Square = 11.62, p = 0.003), as well as depressive disorders by child (Chi Square = 22.18, p ≤ 0.0001) and parent report (Chi Square = 20.45, p < 0.0001). All youth in the anxiety group met diagnostic criteria for an anxiety disorder by child (n = 10) or parent report (n = 9).

Seven youth with CVS met diagnostic criteria for an anxiety disorder by child (n = 5) or parent report (n = 7). One youth in the control condition met criteria for a specific phobia, but was kept in the control condition as the phobia was for worms in a child who lived in a desert climate and didn't encounter worms. Three parents of youth in the control group reported symptoms of an anxiety disorder in their child, but as the child did not endorse anxiety symptoms consistent with these disorders they were left in the control group.

3.2.2. Anxiety symptoms

Thirty-eight youth completed the self-report version of the SCARED and 37 parents completed the parent proxy version. The SCARED demonstrated excellent internal reliability for the youth (Cronbach's α = 0.95) and the parent proxy reports (Cronbach's α = 0.95). There was good agreement between parent and child ratings of the child's anxiety symptoms on the SCARED (ICC = 0.74; 95% CI: 0.46–0.87, p < 0.0001). Significant differences were found for the self-report (F = 27.22, df = 35, 2 p < 0.001) and parent proxy forms (F = 14.92, df = 34, 2 p < 0.001) of the SCARED for youth in the anxiety and CVS groups as compared to the control group. No significant differences were found between the CVS group and the anxiety group for the child or parent proxy forms of the SCARED. No youth in the control group met clinical cut-off for an anxiety disorder by child or parent report. Nine children (82%) in both the anxiety and the CVS groups met clinical cut off for an anxiety disorder by self-report. In contrast 7/10 (70%) of parent proxy reports for the youth in the anxiety group and 4/11 (36%) of parent proxy reports for youth in the CVS group reported symptoms that met clinical cut-off for anxiety symptoms on the SCARED (i.e., total score of ≥25) (Table 2).

Table 1
Number of subjects with DSM-IV psychiatric diagnoses from child and parent ADIS-C interviews.

Group	Child interview	Parent interview
Control	(n = 16)	(n = 14)
	Specific phobia - 1	Social anxiety - 1
		Panic - 2
Anxiety	(n = 11)	(n = 10)
	Specific phobia - 1	Specific phobia - 4
	Social anxiety - 7	Social anxiety - 6
	Generalized anxiety - 9	Generalized anxiety – 7
	Panic - 5	Panic - 2
	OCD - 3	OCD - 0
	PTSD - 2	PTSD - 0
	Major depression - 1	Major depression - 1
	(+8 past history)	(+7 past history)
CVS	(n = 11)	(N = 11)
	Specific phobia - 1	Specific phobia - 0
	Social anxiety - 3	Social anxiety - 3
	Generalized anxiety - 5	Generalized anxiety - 6
	Panic - 1	Panic - 1
	OCD - 0	OCD - 0
	PTSD - 2	PTSD - 1
	Major depression - 2	Major depression - 2
	(+5 past history)	(+6 past history)

3.2.3. State anxiety pre and post TSST-C

The STAI-C demonstrated excellent internal reliability for both the pre TSST-C (Cronbach's α = 0.86) and the post TSST-C measurements (Cronbach's α = 0.90). There were significant differences among the groups with regard to both pre TSST-C state anxiety (F = 5.37, df = 35, 2, p = 0.009) and post TSST-C state anxiety (F = 8.90, df = 34, 2, p = 0.001). Post-hoc analyses of pre TSST-C state anxiety found significant differences between the anxiety and control groups −4.53 (95% CI: −8.29, −0.78) p = 0.01, but no significant differences between the CVS group and the anxiety or the control groups. Post-hoc analyses of post TSST-C state anxiety found significant differences between the anxiety and the control group −6.66 (95% CI: −11.40, −1.92), p = 0.004 and the CVS and the control group −6.43 (95% CI: −11.03, −1.82), p = 0.004 but no significant differences between the CVS and the anxiety groups (Table 3).

Changes in pre to post TSST-C state anxiety scores were examined to evaluate whether the TSST-C produced increases in perceived stress. The differences between pre and post STAI-C scores were significantly increased for the sample as a whole (t = 4.36, df = 36, p < 0.001; Cohen's d = 0.62) and for each of the study groups, CVS (t = 2.90, df = 10, p = 0.016; Cohen's d = 0.87), anxiety (t = 2.28, df = 9, p = 0.048; Cohen's d = 0.74), and control (t = 2.94, df = 15, p = 0.01; Cohen's d = 0.57), with effect sizes from small to large, indicating the TSST-C produced the desired increase in perceived stress.

3.3. Physiological measures

Due to the lower than expected number of participants recruited to the study the combined physiological data for all participant groups was analyzed to evaluate the effect of the TSST-C over time on salivary cortisol, α-amylase and RMSSD as well as for differences among the study groups over the course of the TSST-C.

3.3.1. Study groups combined: cortisol, α-amylase and RMSSD over the course of the TSST-C

There were significant differences between average cortisol values from the baseline to the recovery period (p = 0.0004) and the stress period to the recovery period (p = 0.005) but not between the baseline and stress periods (p = 0.36). Cortisol levels were on average, 0.05 (SE: 0.01) units higher in the recovery period compared to baseline. Cortisol levels were on average, 0.03 (SE: 0.01) units higher in the recovery period compared to the stress test. There were not significant differences in α-amylase values between any of the periods (p > 0.05).

There were significant differences between average RMSSD values from baseline to the stress period (p = 0.0001) and the stress period to the recovery period (p = 0.004) but not between the baseline and recovery period (p = 0.24). RMSSD was lower on average by 7.9 (SE: 2) units during the stress test when compared to baseline. RMSSD was higher on average by 5.6 (SE: 2) units at the recovery period when compared to the stress test (Table 4).

3.3.2. Group differences: cortisol, α-amylase and RMSSD over the course of the TSST-C

The change in cortisol over time was not significantly different among the three study groups. The change in α-amylase from baseline to the recovery period was significantly different between the control and anxiety group; p = 0.01. The difference in α-amylase from baseline

Table 2
SCARED total scores for child self-report and parent proxy-report and comparative analyses across groups[a].

	CVS		Anxiety		Control		Effect size
Scale	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)Child	
Child self-report total	11	28.09 (9.99)	11	38.18 (14.46)	16	10.44 (4.75)**	2.41
Parent proxy-report total	11	21.82 (15.49)	10	30.20 (9.46)	16	7.56 (6.62)*	1.29

[a] Analyses of variance were performed to examine differences between children with CVS and the other groups. The Bonferroni method was used for post-hoc comparisons between the CVS group and the anxiety and control groups. Significant differences between the CVS and other groups are designated by *$p < 0.01$, **$p < 0.001$. Effect sizes are for comparisons between the CVS and the control groups; Cohen's *d* was used to calculate effect sizes, with 0.20–0.30 designated as a small effect, 0.50 a medium effect and 0.80, a large effect.

to the recovery period was 41.5 (SE: 16.1) units higher in the anxiety group compared to the control group. The change in RMSSD over time was not significantly different among the three study groups (Table 5).

As youth with CVS also evidenced significant anxiety symptoms, the role of anxiety symptom burden in relation to cortisol, α-amylase and RMSSD over the course of the TSST-C was examined. We chose the SCARED score, a continuous variable, rather than categorical anxiety diagnoses for this analysis to provide an evaluation of the cumulative impact of anxiety symptoms on the biomarkers. There was not, however, a significant association between the measured biomarkers and baseline anxiety as measured by the Child SCARED Total score; $p > 0.05$. Based on these results anxiety symptom score was not considered as a confounder.

3.4. Associations among pre and post TSST-C state anxiety and cortisol, α-amylase and RMSSD

There were no significant relationships identified between pre and post TSST-C state anxiety and concurrent cortisol or α-amylase measurements for the sample as a whole or for the study groups separately. However, significant associations were found for the sample as a whole between baseline RMSSD and pre-test state anxiety ($r = -0.406$, $p = 0.012$) and between recovery period RMSSD and post-test state anxiety ($r = -0.501$, $p = 0.002$).

4. Discussion

Our study evaluated anxiety and physiological responses during a standardized stress challenge, the TSST-C, in youth with cyclic vomiting syndrome (CVS) and an anxiety disorder compared to healthy controls. There was a very high rate of anxiety disorders in youth with CVS (64%) identified by standardized diagnostic interviews with the parent and child. This rate is considerably higher than the 27–48% reported in prior studies that relied upon self-report questionnaires (Tarbell and Li, 2008; Tarbell and Li, 2015; Withers et al., 1998). However, an even higher percentage of youth met the cut-off for clinical anxiety symptoms by self-report (82%) in the CVS group in this study, which was the same percentage of youth meeting clinical cut off for their anxiety symptoms in the anxiety comparison group. Parent proxy ratings of their child's anxiety on the SCARED were lower for both the anxiety and the CVS groups which is consistent with prior literature related to reports of internalizing symptoms, such as anxiety, by a proxy rater (Wren et al., 2004). The high percentage of youth in this study that met criteria for clinical anxiety disorders may be related to the small sample size and a potential selection bias of those choosing to participate in this study that sought to evaluate the relationship of anxiety to CVS symptoms. A larger sample of CVS subjects would enable a more detailed examination of whether the type of anxiety disorder(s) as well as psychiatric disorder comorbidity or "load" affects CVS outcomes. Nonetheless, the overall the high rate of anxiety disorders in the youth with CVS raises the issue of whether youth with CVS and clinically significant anxiety symptoms constitute a separate phenotype of CVS and require screening to evaluate the role of anxiety in CVS evaluation and management.

Enduring or trait symptoms of anxiety as measured by the youths' SCARED total score were not significantly associated with the biomarkers assessed during the TSST-C. However, state (i.e., acute) anxiety was negatively correlated with HRV measured immediately prior to and after the TSST-C for the sample as a whole. HRV as indexed by RMSSD decreased significantly in response to the stress test and increased during the recovery period for the sample as a whole. This finding is consistent with other studies that have found a large effect for decreases in HRV in children during the TSST regardless of psychopathology (Shahrestani et al., 2014). Our results are similar to those of Alkozei and colleagues (Alkozei et al., 2015) who found increased state anxiety during a laboratory stress challenge to be associated with alterations in cardiac autonomic responding in a cohort of children with anxiety and normal controls. These investigators also found no differences between the control and the anxiety groups in the autonomic measure and no relationship of more enduring anxiety to the autonomic changes. Alkozei et al. (2015) suggested that differences in autonomic regulation found between anxious and control children in prior research may be related to differences in subjective anxiety during the stress task, rather than due to chronic autonomic dysregulation. Several studies have found alterations in autonomic function as indexed by HRV to be associated with a range of childhood psychiatric conditions (Boyce et al., 2001; Srinivasan, 2007).

Findings have been mixed with regard to anxiety disorders with some studies reporting attenuated restorative autonomic function or higher heart rate and lower autonomic parasympathetic arousal in response to stress in children with anxiety disorders compared to normal controls (Boyce et al., 2001; Kramer et al., 2012; Sharma et al., 2011a) while others did not find differences (Kristensen et al., 2014; Sharma et al., 2011b). It is likely that methodological differences, including

Table 3
Pre and post TSST-C state anxiety scores across groups[a].

	CVS		Anxiety		Control		Total		Effect size
State anxiety	N	Mean(SD)	N	Mean(SD)	N	Mean(SD)	N	Mean(SD)	Cohen's *d*
Pre TSST-C score	11	31.91 (4.67)	11	32.91 (3.70)*	16	28.37 (2.89)	38	30.71 (4.24)	Anxiety-control 1.40
Post TSST-C score	11	36.36 (5.52)*	10	36.60 (6.11)*	16	29.94 (2.59)	37	33.65 (5.60)	Anxiety-control 1.53 CVS-control 1.59

[a] Analyses of variance were performed to examine differences among the 3 study groups. The Bonferroni method was used for post-hoc comparisons among the groups. Significant differences between the control and other groups are designated by *$p \leq 0.01$. Effect sizes were calculated when differences between the groups were statistically significant for the post hoc tests. Cohen's *d* was used to calculate effect size, with 0.20–0.30 designated as a small effect, 0.50 a medium effect and 0.80, a large effect.

Table 4
Total sample means: cortisol, α-amylase and RMSSD over the course of the TSST-C.

Time	Baseline	Stress	Recovery
Cortisol	0.11 (0.09, 0.13)	0.12 (0.1, 0.15)	0.16 (0.14, 0.18)
Amylase	95.11 (76.23, 113.98)	97.9 (79.02, 116.77)	95.96 (77.09, 114.84)
RMSSD	45.35 (39.59, 51.12)	37.44 (31.67, 43.2)	43.06 (37.27, 48.85)

sample size, type of stress challenge, comparison groups, anxiety and HRV measures chosen, as well as the age and gender of the subjects all may contribute to these mixed results.

In this pilot study we were not able to control for gender, type and number of anxiety disorders and our sample size was quite small. Thus, our findings regarding the relationship of HRV with state but not trait anxiety need to be interpreted with caution. In addition, given the preliminary evidence of dysautonomia in children with CVS (Chelimsky and Chelimsky, 2007; To et al., 1999), primarily sympathetic autonomic dysfunction, it will be essential to integrate measures of state and trait anxiety as well as autonomic measures, such as HRV, into future studies to better understand the inter relationships among these factors in acute stress responses in youth with cyclic vomiting syndrome.

HRV as indexed by RMSSD was the biomarker most sensitive to changes over the course of the TSST-C for the sample as a whole, with RMSSD decreasing in response to the stress tasks of the TSST-C (public speaking and mental arithmetic) and increasing upon completion of the stress tasks. RMSSD did not however distinguish among the study groups, and this may have been due to the small sample size. It is of interest that the RMSSD for the CVS group was the lowest among the study groups across the 3 time periods of the TSST-C (baseline, stress test, recovery), but these group differences were not statistically significant. Thus, we think it would be worthwhile to evaluate RMSSD in a larger sample to better evaluate potential group differences, which would also allow for a more detailed look at the various components of HRV (i.e., sympathetic to parasympathetic input).

We found a significant increase in cortisol over the course of TSST-C for the 38 subjects, but no differences among the study groups in cortisol response. Cortisol levels were higher on average in the recovery period compared to baseline for the study participants as a whole, which is consistent with the expected increase in cortisol values over time in response to an acute stressor (Kudielka et al., 2007). Significant increases in α-amylase over the course of the TSST-C were found for the anxiety group only. Alpha amylase did not prove to be as sensitive a biomarker for stress response in the study participants. Other studies that have evaluated stress response to the TSST in children have reported more robust changes in α-amylase (Yim et al., 2015). This discrepancy may be related to the small sample size in the current study and the use of the child version of the TSST, which is designed to be a less intense stress challenge than the adult version. The child version was selected due to concern about the potential of the adult TSST to trigger a CVS episode in affected participants, but it may not have been a sufficiently potent a challenge to produce an increase in salivary α-amylase for the adolescents in this study. Further, while the Trier Social Stress Test is one of the most widely used and extensively validated methods for inducing moderate psychosocial stress in laboratory settings in a wide range of populations, particularly for cortisol responses (Allen et al., 2014; Dickerson and Kemeny, 2004; Kudielka et al., 2007) other paradigms and physiological endpoints may need to be considered. For example, cognitive tasks such as the dot-probe that evaluates threat perception (Frewen et al., 2008) or the Stroop test that has been shown to affect subjective perception of threat and changes in heart rate, heart rate variability (Delaney and Brody, 2000) and inhibition of gastric activity (Huerta-Franco et al., 2012) have the potential to broaden our understanding of the stress response in youth with CVS. Functional neuroimaging techniques that investigate neural circuits involved in stress reactivity may also add our understanding of how perceived stress may lower the threshold for CVS episodes.

This study has several limitations. As this was a pilot study, one of our goals was to evaluate the feasibility of recruiting participants in a reasonable time period. While we were able to recruit 84% of the planned number of participants, it took four years to enroll 73% (11/15) of the desired number of eligible youth with CVS. In spite of the multiple methods used to facilitate participation, including clinic visits, website announcement, blast emails and community postings we were unable to meet our recruitment goals, which limits the power of the study to detect differences among the study groups. Further, based on the study's stated goal, there is the possibility that a selection bias occurred in which CVS subjects with anxiety were disproportionately included. Future studies involving multiple clinic sites including those specializing in CVS are needed to provide sufficient power to optimally evaluate differences among children with CVS, with and without comorbid anxiety. Regarding methodology, we asked our participants not to eat or drink 1 h prior to the TSST-C, which was scheduled in the later afternoon. This may have led to less robust changes in cortisol over the course of the TSST-C, as prior research has indicated that low glucose levels may inhibit adrenocortical responsiveness (Kirschbaum et al., 1997). Finally, we were unable to control the medications used by the participants. While a preliminary analysis indicated that SSRIs and tricyclic antidepressants did not impact the biomarkers for the Anxiety and CVS groups, this finding needs to be interpreted cautiously given the small number of subjects on these medications. Control of medications that could impact physiological outcomes would allow for more definitive characterizations of stress response in youth with CVS, however withdrawal of medications that may be keeping symptoms under control presents additional challenges.

In summary, this is the first study to examine whether stress, the most commonly reported trigger for CVS episodes, is associated with changes in HPA axis activation and autonomic responses in youth with CVS. We found high rates of anxiety both by diagnostic interview and parent and child report confirming and expanding our understanding of the comorbidity of CVS with anxiety symptoms in adolescents. We identified state anxiety to be inversely related to HRV during acute stress, which warrants further study to evaluate the role of acute anxiety as a potential proximal mechanism by which a stressor might

Table 5
Group means: cortisol, α-amylase and RMSSD over the course of the TSST-C[a].

Group	Time	Control (n = 16)	Anxiety (n = 11)	CVS (n = 11)
Cortisol	Baseline	0.12 (0.1, 0.15)	0.10 (0.08, 0.13)	0.11 (0.08, 0.14)
	Stress	0.13 (0.1, 0.17)	0.12 (0.09, 0.16)	0.11 (0.09, 0.14)
	Recovery	0.15 (0.08, 0.22)	0.17 (0.12, 0.22)	0.16 (0.1, 0.21)
Amylase	Baseline	105.65 (78.15, 133.15)	82.72 (46.94, 118.49)	92.16 (53.23, 131.09)
	Stress	104.75 (75.08, 134.43)	89.39 (47.97, 130.81)	96.43 (53.67, 139.19)
	Recovery	90.95 (65.43, 116.46)	109.5 (52.04, 166.96)	89.72 (49.37, 130.07)
RMSSD	Baseline	50.57 (40.37, 60.78)	44.5 (29, 59.99)	38.61 (28.8, 48.43)
	Stress	39.87 (31.95, 47.79)	37.09 (27.94, 46.25)	34.25 (21.7, 46.8)
	Recovery	46.9 (36.76, 57.04)	43.34 (30.31, 56.37)	34.12 (24.59, 43.66)

[a] The change in α-amylase from baseline to the recovery period was significantly different between the control and anxiety group; p = 0.01. No significant group differences by time period were found for cortisol or RMSSD.

trigger a CVS episode. Further research is indicated to better characterize both psychological and physiological responses to stress in youth with CVS to more accurately capture the complex interrelationships among anxiety symptoms, physiological reactivity and CVS episodes. Ambulatory monitoring of the psychological and physiological responses to acute stressors in real life settings, as could be achieved via momentary assessment of daily stressors (Smyth et al., 1998) may afford a more detailed characterization of stress responses in youth with CVS. The insights derived from such studies have the potential to contribute to a better understanding of the pathophysiology of CVS and most importantly, to inform and improve our treatments for this highly aversive and often disabling condition.

5. Conclusion

Anxiety was found to be prevalent in pediatric CVS, and thus screening for anxiety in pediatric CVS patients is indicated given its significant comorbidity. HRV was the most sensitive measure of physiological reactivity to the standardized stressor for the study participants. The inverse associations found between HRV and state anxiety during the TSST-C suggest that it may be a useful biomarker for future studies evaluating stress as a potential trigger for CVS episodes. The finding that state anxiety but not trait anxiety was associated with changes in HRV, suggests that acute changes in anxiety may be more relevant to investigation of the linkage between stress and CVS episodes than more enduring or trait anxiety.

Conflict of interest

None.

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References

Abu-Arafeh, I., Russell, G., 1995. Cyclical vomiting syndrome in children: a population based study. J. Pediatr. Gastroenterol. Nutr. 21, 454–458.
Alkozei, A., Creswell, C., Cooper, P., Allen, J., 2015. Autonomic arousal in childhood anxiety disorders: associations with state anxiety and social anxiety disorder. J. Affect. Disord. 175, 25–33. http://dx.doi.org/10.1016/j.jad.2014.11.056.
Allen, A., Kennedy, P., Cryan, J., Dinan, T., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci. Biobehav. Rev. 38, 94–124.
Birmaher, B., Brent, D., Chiappetta, L., Bridge, J., Monga, S., Baugher, M., 1999. Psychometric properties of the Screen for Child Anxiety Related Emotional Disorders (SCARED): a replication study. J. Am. Acad. Child Adolesc. Psychiatry 38, 1230–1236.
Boyce, W., Quas, J., Alkon, A., Smider, N., Essex, M., Kupfer, D., 2001. Autonomic reactivity and psychopathology in middle childhood. Br. J. Psychiatry 179, 144–150.
Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., Hellhammer, D., 1997. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. Psychosom. Med. 59, 419–426.
Chatterton, R.T., Vogelsong, K.M., Lu, Y., Ellman, A.B., Hudgens, G.A., 1996. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin. Physiol. 16, 433–448.
Chelimsky, T., Chelimsky, G., 2007. Autonomic abnormalities in cyclic vomiting syndrome. J. Pediatr. Gastroenterol. Nutr. 44, 326–330. http://dx.doi.org/10.1097/MPG.0b013e31802bddb7.
Delaney, J., Brody, D., 2000. Effects of short-term psychological stress on the time and frequency domains of heart-rate variability. Percept. Mot. Skills 91, 515–524.
Dickerson, S., Kemeny, M., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.
Dieleman, G., Huizink, A., Tulen, J., Utens, E., Creemers, H., van der Ende, J., Verhulst, F., 2015. Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis. Psychoneuroendocrinology 51, 135–150. http://dx.doi.org/10.1016/j.psyneuen.2014.09.002.
Dorn, L., Campo, J., Thato, S., Dahl, R., Lewin, D., Chandra, R., Di Lorenzo, C., 2003. Psychological comorbidity and stress reactivity in children and adolescents with recurrent abdominal pain and anxiety disorders. J. Am. Acad. Child Adolesc. Psychiatry 42, 66–75. http://dx.doi.org/10.1097/00004583-200301000-00012.
Drumm, B.R., Bourke, B., Drummond, J., McNicholas, F., Quinn, S., Broderick, A., ... Rowland, M., 2012. Cyclical vomiting syndrome in children: a prospective study. Neurogastroenterol. Motil. 24, 922–927. http://dx.doi.org/10.1111/j.1365-2982.2012.01960.x.
Ertekin, V., Selimoglu, M.A., Altnkaynak, S., 2006. Prevalence of cyclic vomiting syndrome in a sample of Turkish school children in an urban area. J. Clin. Gastroenterol. 40, 896–898.
Forbes, D., Withers, G., Silburn, S., McKelvey, R., 1999. Psychological and social characteristics and precipitants of vomiting in children with cyclic vomiting syndrome. Dig. Dis. Sci. 44, 19S–22S.
Frewen, P., Dozois, D., Joanisse, M., Neufeld, R., 2008. Selective attention to threat versus reward: meta-analysis and neural network modeling of the dot-probe task. Clin. Psychol. Rev. 28, 307–337.
Gaab, J., Blattler, T., Menzi, T., Pabst, S., Stoyer, S., Ehlert, U., 2003. Randomized controlled evaluation of the effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects. Psychoneuroendocrinology 28, 767–779.
Huerta-Franco, M., Vargas-Luna, M., Montes-Frausto, J., Morales-Mata, I., Ramirez-Padilla, L., 2012. Effect of psychological stress on gastric motility assessed by electrical bioimpedance. World J. Gastroenterol. 18, 5027–5033.
Kirschbaum, C., Gonzalez Bono, E., Rohleder, N., Gessner, C., Pirke, K., Salvador, A., Hellhammer, D., 1997. Effects of fasting and glucose load on free cortisol responses to stress and nicotine. J. Clin. Endocrinol. Metab. 82, 1101–1105.
Kramer, M., Seefeldt, W., Heinrichs, N., Tuschen-Caffier, B., Schmitz, J., Wolf, O., Blechert, J., 2012. Subjective, autonomic, and endocrine reactivity during social stress in children with social phobia. J. Abnorm. Child Psychol. 40, 95–104.
Kristensen, H., Oerbeck, B., Torgersen, H.S., Hansen, B.H., Wyller, V.B., 2014. Somatic symptoms in children with anxiety disorders: an exploratory cross-sectional study of the relationship between subjective and objective measures. Eur. J. Child Adolesc. Psychiatry 23, 795–803. http://dx.doi.org/10.1007/s00787-013-0512-9.
Kudielka, B., Hellhammer, D., Kirschbaum, C., 2007. Ten years of research with the Trier Social Stress Test-Revisited. In: Harmon-Jones, E., Winkielman, P. (Eds.), Social Neuroscience. Guildford Press, New York, pp. 56–83.
Li, B., Murray, R., Heitlinger, L., Robbins, J., Hayes, J., 1999. Is cyclic vomiting syndrome related to migraine? J. Pediatr. 134, 567–572.
Li, B., Lefevre, F., Chelimsky, G., Boles, R., Nelson, S., Lewis, D., ... Rudolph, C., 2008. North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition consensus statement on the diagnosis and management of cyclic vomiting syndrome. J. Pediatr. Gastroenterol. Nutr. 47, 379–393.
Sgoifo, A., Braglia, F., Cosstoli, T., Musso, E., Meerlo, P., Ceresini, G., Troisi, A., 2003. Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile. Neurosci. Biobehav. Rev. 27, 179–188.
Shahrestani, S., Stewart, E., Quintana, D., Hickie, I., Guastella, A., 2014. Heart rate variability during social interactions in children with and without psychopathology: a meta-analysis. J. Child Psychol. Psychiatry 55, 981–989.
Sharma, R.K., Balhara, Y.P., Sagar, R., Deepak, K.K., Mehta, M., 2011a. Heart rate variability study of childhood anxiety disorders. J. Cardiovasc. Dis. Res. 2, 115–122. http://dx.doi.org/10.4103/0975-3583.83040.
Sharma, R.K., Sagar, R., Deepak, K.K., Mehta, M., Balhara, Y.P., 2011b. Clinical and autonomic functions: a study of childhood anxiety disorders. Ann. Saudi Med. 31, 250–257. http://dx.doi.org/10.4103/0256-4947.81533.
Silverman, W.K., Albano, A.M., 2004. Anxiety Disorders Interview Schedule (ADIS-IV) Child and Parent Schedules. Oxford University Press, New York.
Smyth, J., Ockenfels, M., Porter, L., Kirschbaum, C., Hellhammer, D., Stone, A., 1998. Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. Psychoneuroendocrinology 23, 353–370.
Speilberger, C., Edwards, C.D., Lushene, J., Montouri, J., Platzek, D., 1973. The State-Trait Anxiety Inventory for Children (STAIC). Consulting Psychologists Press, Palo Alto, CA.
Srinivasan, K., 2007. Childhood psychopathology and autonomic dysregulation: exploring the links using heart rate variability. J. Indian Assoc. Child Adolescen Mental Health 3, 96–104.
Stickler, G.B., 2005. Relationship between cyclic vomiting syndrome and migraine. Clin. Pediatr. 44, 505–508.
Tarantino, S., Capuano, A., Torriero, R., Citti, M., Vollono, C., Gentile, S., ... Valeriani, M., 2014. Migraine equivalents as part of migraine syndrome in childhood. Pediatr. Neurol. 51, 645–649. http://dx.doi.org/10.1016/j.pediatrneurol.2014.07.018.
Tarbell, S., Li, B., 2008. Psychiatric symptoms in children and adolescents with cyclic vomiting syndrome and their parents. Headache 48, 259–266. http://dx.doi.org/10.1111/j.1526-4610.2007.00997.x.
Tarbell, S., Li, B., 2015. Anxiety measures predict health-related quality of life in children and adolescents with cyclic vomiting syndrome. J. Pediatr. 167, 633–638.
To, J., Issenman, R.M., Kamath, M.V., 1999. Evaluation of neurocardiac signals in pediatric patients with cyclic vomiting syndrome through power spectral analysis of heart rate variability. J. Pediatr. 135, 363–366.
Withers, G., Silburn, S., Forbes, D., 1998. Precipitants and aetiology of cyclic vomiting syndrome. Acta Paediatr. 87, 272–277.
Wren, F.J., Bridge, J.A., Birmaher, B., 2004. Screening for childhood anxiety symptoms in primary care: integrating child and parent reports. J. Am. Acad. Child Adolesc. Psychiatry 43, 1364–1371. http://dx.doi.org/10.1097/01.chi.0000138350.60487.d3.
Yim, I., Quas, J., Rush, E., Granger, D., Skoluda, N., 2015. Experimental manipulation of the Trier Social Stress Test-Modified (TSST-M) to vary arousal across development. Psychoneuroendocrinology 57, 61–71.