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Social Anxiety and the Cortisol Response to Social Evaluation in Children and Adolescents

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Abstract

Contradictory findings have been reported on the relation between social anxiety and the cortisol response to social evaluation in youth. The present longitudinal study aimed to clarify this relation by taking pubertal development into account. Data were collected in two waves, two years apart, for a community sample of 196 participants, aged 8-17 years at Time 1. Pubertal development and social anxiety were assessed with self-report questionnaires. Salivary cortisol was obtained before and after participants completed the Leiden Public Speaking Task. Data were analyzed using regression analysis with clustered bootstrap. The dependent variable was the cortisol area under the curve. Social anxiety and pubertal development scores were decomposed into between- and within-participants components. Between participants, the relation between social anxiety and the cortisol response to public speaking varied with pubertal development: socially anxious individuals showed higher responses at low levels of pubertal development, but lower responses at high levels of pubertal development. Within participants, an increase in social anxiety over time was associated with a lower cortisol response. The results are in line with the suggestion that the responses of socially anxious individuals change from elevated in childhood to attenuated in adolescence and adulthood. Attenuation of the cortisol response is explained by theories proposing that the stress response changes with the duration of the stressor.

Keywords: social anxiety, salivary cortisol, social evaluation, pubertal development, adolescence, attenuation
1. Introduction

Social Anxiety Disorder (SAD) is characterized by strong fear or anxiety about social situations in which one may be scrutinized by others (APA, 2013). These fears vary along a continuum in the general population (Rapee & Spence, 2004). For adolescents with SAD or subclinical levels of social fears, public speaking is among the most commonly feared situations (Essau et al., 1999; Gren-Landell et al., 2009). Therefore, it has long been hypothesized that high socially anxious youth show larger cortisol responses to public speaking than low socially anxious youth. Van West et al. (2008) provided support for this hypothesis, but other studies found no difference (Martel et al., 1999; Schmidt et al., 1999; Miers et al., 2011; Kraemer et al., 2012). Based on their review of physiological and neuroendocrine responses in socially anxious youth, Siess et al., (2014) suggested a developmental change from elevated responses in children to no difference and possibly attenuated responses in adolescents and adults. However, the studies available for review were mainly cross-sectional studies on physiological responses. To our knowledge, the current study presents the first longitudinal data on the relation between social anxiety and the cortisol response to public speaking in late childhood to adolescence.

Evidence for a shift from hypercortisolism in childhood to hypocortisolism in adolescence and adulthood has been provided by a few longitudinal studies on (general) internalizing problems and adverse life events. Recent appearance of internalizing symptoms predicted higher diurnal cortisol levels (Ruttle et al., 2011) and elevated responses to a social stress task in adolescence (Booij et al., 2013), whereas a history of internalizing problems predicted attenuated cortisol levels and responses (Booij et al., 2013; Ruttle et al., 2011). Moreover, a study by Trickett et al. (2010) provided evidence for a change within individuals. Female victims of childhood sexual abuse showed higher diurnal cortisol levels than control participants who were not sexually abused shortly after the abuse was disclosed. Sixteen years later, however, the victims showed lower cortisol levels than the control participants. These studies concluded that the time since stressor onset predicts the occurrence of hyper- or hypocortisolism. This conclusion was supported by a meta-analysis of predominantly adult
Van den Bos et al. studies, demonstrating elevated cortisol levels for recent stressors and increasingly attenuated cortisol levels with time since stressor onset (Miller et al., 2007).

Theoretically, attenuated cortisol levels and responses have been interpreted as adaptations to a history of many stressful events: to diminish allostatic load resulting from high cortisol levels (Fries et al., 2005), as adaptive calibration of the stress system to dangerous environments (Del Giudice et al., 2011), and through protective inhibition of motivation to engage in challenges that may exceed one’s resources or induce allostatic load (Tops et al., 2015; Tops et al., 2016).

Attenuation is often related to traumatic events (e.g. Miller et al., 2007; Trickett et al., 2010). The Protective Inhibition of Self-regulation and Motivation (PRISM) model (Tops et al., 2015; Tops et al., 2016) explains how attenuation can result from an accumulation of daily stressors.

Tops et al. (2014) proposed that behavior and homeostasis are controlled prospectively in predictable, safe situations and reactively in unpredictable, threatening situations. Because reactive control takes over when routinized, efficient responses cannot be applied and it is uncertain which responses are optimal, this control is associated with emergency (“just in case”) high-intensity responses and allostatic load. PRISM serves to limit the time spent in reactive control mode (Tops et al., 2015; Tops et al., 2016). It does so by reducing motivation, through adaptation of one’s subjective estimates of efforts needed to meet a challenge, efforts made and resources available (Tops et al., 2015; Tops et al., 2016). The PRISM model predicts that an initially high cortisol response to unpredictable, threatening situations turns into a low cortisol response when the situation persists (Tops et al., 2015).

Cortisol is involved in the regulation of energy for the activation of physiological and neural systems to actively deal with challenges (Sapolsky et al., 2000). For instance, cortisol responses to a public speaking task produce a bias in cognitive processes towards stimuli associated with the challenge (Smeets et al., 2007; c.f. Joëls et al., 2006). The appraisal that overcoming the challenge through active coping is impossible or disproportionately effortful tends to result in low cortisol responses (Denson et al., 2009; Moons et al., 2010). Consequently, low cortisol is associated with
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fatigue, reduced motivation and perceived uncontrollability (Chida & Steptoe, 2009; Tops et al., 2016). Chronic or recurrent challenges are uncontrollable in the sense that, over time, coping attempts that activated physiological and neural systems have proven futile in overcoming them. Socially anxious individuals may often experience a threat of negative evaluation in everyday social situations and this may activate PRISM. Persistently high (or increasing) levels of social anxiety may therefore be associated with a low cortisol response to social evaluation (over time).

In adolescence, the cortisol response is not only affected by stressor history, but also by normative development. Several studies have demonstrated an increase in the cortisol response to social evaluation in adolescence (Klimes-Dougan et al., 2001; Gunnar et al., 2009; Stroud et al., 2009; Sumter et al., 2010). This normative increase appears more strongly related to pubertal development than to age (Van den Bos et al., 2014).

The present longitudinal study investigated whether social anxiety is related to attenuation of the cortisol response to public speaking in a community sample of children and adolescents. We also investigated the interaction between social anxiety and pubertal development, because PRISM may occur alongside the typical puberty-related increase of the cortisol response. We expected the cortisol response to our social-evaluative situation to be negatively related to social anxiety (between and within-participants). However, this relation may only become apparent with advancing pubertal development. Depression and a general measure of anxiety were included as control variables to check whether the relation is specific to social anxiety.

2. Method

2.1 Participants

The data were collected as part of the Social Anxiety and Normal Development (SAND) study, which was approved by the Leiden University Medical Ethical Committee and carried out in accordance with the Declaration of Helsinki. Parents provided active consent; written assent was obtained from participants themselves.
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The SAND-study aimed for a normative sample. Participants were recruited through two primary schools and one secondary school in a middle-sized city in the Netherlands. Students with severe psychological problems or physical illness were excluded from participation. If such problems had been registered at school, students were not invited. To identify individuals with conditions unknown to the school, participants completed a health and medication history questionnaire probing for treatment by a mental health professional and physical complaints. All eligible children in the last three primary school grades were invited to participate. Pupils in the first four grades of secondary school completed the Social Anxiety Scale for Adolescents (SAS-A; La Greca & Lopez, 1998). Then, stratified random sampling was used to invite 204 participants (out of 488), with equal numbers of boys and girls of each age. This procedure resulted in a sample of 126 primary school participants and 173 secondary school participants that was representative of the general population. To address questions related to social anxiety, all remaining secondary school students with standardized SAS-A scores among the top 20% of their sex (n = 38) were invited to participate. The present study used the total sample, with oversampling of high social anxiety levels. Table 1 provides social background characteristics of the participants.

The SAND-study had a cohort-sequential design. Data were collected in four waves, starting in 2006-2007. The public speaking task was administered in Wave 1 and Wave 3, i.e., Time 1 and Time 2 in this study. At Time 1, the task was administered to 327 participants (167 males: 51.1%). Their ages ranged from 8 - 17 years ($M = 13.3, SD = 2.3$, median = 13.3). At Time 2, two years later, 243 participants returned to perform the task again (51.4% male, mean age = 15.2, $SD = 2.2$). The attrition rate was 25.7%. There was no difference in the distribution of sex ($\chi^2(1) = .052, p = .820$) between those who continued to participate and those who did not. Likewise, there was no difference in mean age ($t(325) < 1$), mean score on the Pubertal Development Scale ($t(313) < 1$), cortisol response ($t(308) < 1$), or mean score on the SAS-A ($t(322) = 1.279, p = .202$).

2.2 Procedure
The Leiden Public Speaking Task (LPST; Westenberg et al., 2009) was specifically designed for longitudinal studies: to enhance reproducibility, participants are informed a week beforehand that they have to give a 5-minute speech on movies they like or dislike. This procedure has ecological validity, enhances comparability of the two waves and reduces the risk of confounding the cortisol response to public speaking with a response to the unfamiliar situation.

The task was modeled on a classroom presentation, which all participants had experience with. Participants deliver their speech in front of a projection screen displaying a life-size audience of age peers and a female teacher, who behave neutrally. They are informed that the audience is prerecorded and that their performance will be recorded and evaluated by peers afterwards (e.g., reported in Blöte et al., 2012). This situation of ambiguous rather than negative social evaluation may be particularly suitable to reveal individual differences in sensitivity to social-evaluative threat. As participants cannot directly control how their performance is evaluated, the LPST combines the two characteristics of laboratory procedures that most consistently trigger responses by the Hypothalamic-Pituitary-Adrenal (HPA) axis: social-evaluative threat and uncontrollability (Dickerson & Kemeney, 2004).

Because participants knew beforehand that they would have to give a speech, the pre-task concentration is not a valid baseline. The concentration in the last sample, which is the best indication of a participant’s resting level in this procedure, is used instead. Previous research indicated that the cortisol increase over this concentration in the LPST is comparable with the increase over baseline levels in other youth studies using impromptu procedures such as the Trier Social Stress test (see Westenberg et al., 2009).

At both Time 1 and Time 2, there were two lab-sessions with a one-week interval. Participants were allowed to come in pairs (together with a classmate), but they were tested individually in separate rooms. In the first session, participants performed tests measuring cognitive and psychosocial development and completed self-report questionnaires. This session also served to familiarize participants with the lab and inform them about the public speaking task. They received
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instructions to prepare themselves as they would for a presentation at school. They were also
instructed to refrain from exercising, smoking, eating and drinking caffeinated beverages, dairy
products and alcohol one hour before the start of the public speaking session. The second session
consisted of a pre-task resting period (25 min), the public speaking task (15 min) and a post-task
recovery period (30 min). All sessions at Time 1 and Time 2 started at 2:15 p.m. to minimize diurnal
effects.

2.3 Measures

2.3.1 HPA-axis activity. For the assessment of cortisol (nmol/l), seven saliva samples were
collected by passively drooling into plastic vials (IBL-SaliCap®, Germany) directly or through a straw.
Sample 1 was taken after the resting period. Six samples were taken after the speech to account for
individual differences in the timing of the cortisol response. Sample 2 was collected directly after the
speech. Sample 3 was taken 10 minutes later. Samples 4 to 7 were collected at intervals of 5 minutes,
so that the last saliva sample was taken at the end of the recovery period. Figure 1 illustrates the
timing of the saliva samples.

The determination of cortisol in saliva was performed with a competitive
electrochemiluminescence immunoassay ECLIA using a Modular Analytics E170 immunoassay
analyzer from Roche Diagnostics (Mannheim, Germany). The sample volume was at least 20 μl.
Cortisol concentrations were only determined for samples from participants assessed at both times.
Samples from one participant at one time of measurement were batched together for analysis. The
inter-assay coefficient of variability was 10.1%. Missing values due to insufficient volume ranged
between 0.4 and 13.6% of the samples (M = 4.6%). Three values at Time 1 (96 nmol/l in sample 2,
168 nmol/l in sample 3, 179 nmol/l in sample 7) and 5 values at Time 2 (76 and 107 nmol/l in sample
1, 79 nmol/l in sample 3, 85 nmol/l in sample 5, 102 nmol/l in sample 7) were treated like missing
values, because the samples were likely contaminated by blood.

2.3.2 Visual Analog Scale Nervousness. Subjectively experienced nervousness was assessed
with the VAS (Davey et al., 2007). Participants marked a point representing their nervousness on a 10
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The distance was measured in mm. They reported how nervous they felt at four times: after the nature video, after the speech, during the speech (retrospectively) and after the recovery period.

**2.3.3 Social Anxiety Scale for Adolescents.** Social anxiety was measured with the Dutch translation (Koot & Utens, unpublished) of the SAS-A (La Greca & Lopez, 1998). The test consists of 18 self-descriptive statements related to social anxiety and 4 filler items. An example of a test item is: “I worry about what others think of me”. Participants have to indicate on a 5-point scale how much each item is true for them (1 = “not at all”, 5 = “all the time”). Sum scores ranged from 18 - 83 at Time 1 (N = 325) and from 18 - 86 at Time 2 (N = 243). The mean rating over all 18 test items was used in the analyses. Cronbach’s α was .94 at Time 1 and .93 at Time 2.

**2.3.4. Fear Survey Schedule for Children Revised.** The Dutch version (Oosterlaan et al., 1995) of the FSSCR (Ollendick, 1983) was used as a general measure of (trait) anxiety. The test consists of a list of 80 potentially fearful objects and situations (social and non-social). Participants rated their fearfulness on a 4-point scale (1 = “not fearful”, 4 = “very fearful”). Sum scores ranged from 80 – 236 at Time 1 (N = 326) and from 80 - 244 at Time 2 (N = 248). Cronbach’s α was .96 at both times.

**2.3.5 Children’s Depression Inventory.** Level of depression was measured with the Dutch translation (Timbremont & Braet, 2002) of the CDI (Kovacs, 1992). The CDI is a self-report questionnaire of 27 items, designed for children of 8 - 17 years (Timbremont & Braet, 2002). In this study only 26 items were administered, because one item about suicidal ideation was considered inappropriate for our community sample. Each item consists of three statements (in random order): one indicating absence of a symptom (score 0), one indicating a mild symptom (score 1) and one indicating a clinically significant symptom (score 2). Participants are instructed to mark the statement that best reflects their thoughts and feelings over the last two weeks for each item. Item scores are summed to compute the total score, potentially ranging from 0 - 52 in the present study (Time 1: 0-31, N = 324; Time 2: 0-28, N = 242). Cronbach’s α was .80 at Time 1 and .84 at Time 2, demonstrating adequate internal consistency with 26 items.
2.3.6 Pubertal Development Scale. Self-reported pubertal development was measured with a Dutch translation of the PDS (Petersen et al., 1988). A pubertal development score was computed over three items for each sex: menarche, pubic hair development and breast development for girls; voice change, pubic hair development and facial hair development for boys. These items are the ones on which pubertal status categories are based according to the PDS manual (Crockett, 1988) because they are more reliable than the items concerning skin change and growth spurt (Petersen et al., 1988). The individual items were scored on a scale from 1 to 4, except for the item concerning menarche, which was scored as 1 if the girl had not experienced menarche yet and as 4 if she had (Petersen et al., 1988). The overall pubertal development score was calculated by averaging the ratings on the three items. At Time 1, 315 participants completed the PDS (boys: $M = 2.1, SD = 0.9$; girls: $M = 2.8, SD = 1.1$). Cronbach’s alpha was .85 for boys and girls. At Time 2, 242 participants completed the PDS (boys: $M = 2.7, SD = 1.0$; girls: $M = 3.4, SD = .09$). Cronbach’s alpha was .88 for boys and .83 for girls.

2.3.7 Treatment of other factors potentially influencing cortisol concentrations. At the beginning of the public speaking session, participants completed a questionnaire on factors potentially influencing the cortisol concentration, including current medication usage, eating and drinking less than one hour ago and current phase of the menstrual cycle and use of oral contraceptives in girls. We also registered whether participants were tested alone (Time 1: 20.4%, Time 2: 34.2%) or simultaneously with another participant in a different room. Long-term use of medication was assessed with a health and medication history questionnaire filled out at home. Some participants used medication for allergies (6 at Time 1, 9 at Time 2), asthma (9 at Time 1, 5 at Time 2), inflammation (3 at Time 2), ADHD (2 at Time 1, 4 at Time 2), painkillers (3 at Time1, 8 at Time 2) and various other medications (5 at Time 1, 3 at Time 2). Use of any medication, eating or drinking milk less than one hour before the session (8 at Time 1, 7 at Time 2), use of oral contraceptives, and being tested alone were statistically controlled for in the main analyses. All
significant effects remained when participants using a type of medication or consuming food were excluded (see Supplementary Materials).

We did not control for phase of the menstrual cycle. In adult studies, this variable is often used to control for fluctuations in estradiol, which affect the cortisol response. However, the variable is of limited use in adolescents. Fluctuations in estradiol begin years before menarche, whereas the phase of the menstrual cycle can only be determined once a girl has developed a regular cycle (Shirtcliff et al., 2009). In the present study, the phase of the menstrual cycle could not be determined for the majority of girls. At Time 1, 46% was pre-menarche, 22% had an irregular cycle, 11% used oral contraceptives and 3% inadequately reported the time since their last period (9% follicular phase, 9% luteal phase). At Time 2, 27% was pre-menarche, 18% had an irregular cycle, 27% used oral contraceptives and 6% inadequately reported the time since their last period (10% follicular phase, 12% luteal phase).

2.4 Statistical analyses

The main dependent variable was the cortisol response, as measured by the Area Under the Curve with respect to increase (AUCi; Pruessner et al., 2003). AUCi is an index of the overall stress response, sensitive to both its height and its duration. It represents the increase in concentration relative to a baseline. With the LPST, the reference sample is the last sample (7). AUCi was computed over the available samples for each individual, unless the concentration in the first or last sample was missing. For each sample, outliers of more than 3SD were winsorized.

The analyses were done on cases with complete data at both Time 1 and Time 2. As a first step, separate regression analyses were done for each time point (in SPSS 23). The data contained 4 multivariate outliers resulting in large negative AUCis at Time 1 (-96, -86, -81, -80) and 5 at Time 2 (-756, -152, -146, -119, -94). These cases were excluded to meet the assumptions of regression analysis (n = 196). Additionally, analyses were performed on the natural logarithm of AUCi to meet the linearity assumption. Three different models were tested. Model 1 included the explanatory

\[ 1 \] Because the transformation can only be applied to positive numbers, a constant of 57 was added to each AUCi at Time 1 and Time 2, resulting in a minimum (untransformed) value of 1.
variables of interest, PDS-score, SAS-score and their interaction, and the dummy coded control variables female sex, use of medication, use of oral contraceptives, recent food intake, and being tested alone. Model 2 also included the natural logarithm of the cortisol concentration in the reference sample (7). With this model, we controlled for the possibility that social anxiety and pubertal development only predicted AUCi because of a relation with the concentration in the reference sample. Model 3 included the FSSCR-score and the CDI-score as additional control variables to investigate whether the effects were specific to social anxiety.

Second, the data from Time 1 and Time 2 were combined to investigate the effects of changes over time. These analyses were done in R 2.5.1. (R development core team, 2007). Regression analysis with clustered bootstrap (Cameron et al., 2008; Harden, 2011) was used, because this technique is suitable for time-varying predictors. It allows for controlling the cortisol response at Time 1 for control variables at Time 1 and the cortisol response at Time 2 for control variables at Time 2. Intercepts and regression weights were estimated as in standard regression analysis, but the clustered bootstrap procedure (Sherman & Le Cessie, 1997) was used for statistical inference (i.e., standard errors were derived by bootstrapping). From the total data set, 10,000 bootstrap samples of the same size as the original set were drawn randomly with replacement. To deal with the dependency between measurements of the same individual, the bootstrap was clustered: individuals were sampled rather than cases, so that both measurements were included in the sample.

In the analysis over time, PDS- and SAS-scores were decomposed into initial levels and change components (Hedeker & Gibbons, 2006; c.f. Van den Bos et al., 2014). The initial levels (T1PDS, T1SAS) are measures of the between-participants effects. The change components (current score minus score at Time 1; ΔPDS, ΔSAS) are measures of the within-participants effects. The explanatory variables of interest were T1PDS, ΔPDS, T1SAS, ΔSAS, T1PDS x T1SAS, T1PDS x ΔSAS, ΔPDS x T1SAS and ΔPDS x ΔSAS. As before, female sex, use of medication, use of oral contraceptives, recent food intake, and being tested alone were included as control variables in Model 1. The cortisol
concentration in the reference sample was included as an additional control variable in Model 2. FSSCR and CDI were included as additional control variables in Model 3.

Due to collinearity with PDS, age could not be added as a control variable. Models with age instead of PDS showed similar patterns of results, but had a poorer fit to the data ($QIC_{M1} = 779.8$, $QIC_{M2} = 777.4$, $QIC_{M3} = 781.3$). Two-way and three-way interactions with sex were not significant. (Models not reported.)

3. Results

Repeated measures ANOVAs were done to investigate whether self-reported nervousness and the cortisol concentration were affected by the LPST. VAS nervousness ratings were significantly affected by the task at Time 1, Wilks Λ = .176, $F(2,195) = 456.91$, $p < .001$, $η^2_p = .824$, and Time 2, Wilks Λ = .264, $F(2,195) = 271.82$, $p < .001$, $η^2_p = .736$. Participants reported being significantly more nervous during the speech (Time 1 $M = 60.9$, Time 2 $M = 51.2$) than before (Time 1 $M = 36.4$, Time 2 $M = 34.3$) or after (Time 1 $M = 7.2$, Time 2 $M = 6.9$). Likewise, repeated measures ANOVAs on the cortisol concentrations in the 7 saliva samples showed significant effects of sample at Time 1, Wilks Λ = .518, $F(6, 155) = 24.08$, $p < .001$, $η^2_p = .482$, and Time 2, Wilks Λ = .557, $F(6, 190) = 25.22$, $p < .001$, $η^2_p = .443$. Figure 1 illustrates how the mean cortisol concentration changes over the 7 samples at Time 1 and Time 2. At Time 1, 61% of participants showed an increase of at least 2.5 nmol/l over the reference sample (7). At Time 2, the response rate was 71%. Table 2 provides means, standard deviations and correlations between variables. Social anxiety correlated positively with self-reported nervousness during the speech at both times. Correlations over time for the main variables were $r_{PDS} = .825$, $r_{SAS} = .523$ and $r_{AUCi} = .230$ (all $p’s ≤ .001$).

3.1 Social Anxiety and the Cortisol Response at Time 1

Model 1 was significant, $F(8,187) = 7.73$, $p < .001$, $R^2 = .249$. See Table 3 for regression weights. AUCi was positively related to PDS. Moreover, the PDS x SAS interaction was significant. Compared to participants with low SAS-scores, the cortisol responses of participants with high SAS-scores were higher at low levels of pubertal development, but lower at high levels of pubertal
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development (see Figure 2). Model 2 explained more variance than Model 1, $R^2 = .260$, $F_{\text{change}}(1, 186) = 2.79$, $p = .097$ at trend-level. The cortisol concentration in the reference sample was positively associated with AUCi. Importantly, the effects of PDS and PDS x SAS remained significant, indicating that they were significantly related to AUCi independent of possible effects on the reference concentration. An additional regression analysis, predicting the natural logarithm of the cortisol concentration in the reference sample from Model 1, indicated that the reference concentration was in fact unrelated to PDS and SAS: the regression model was not significant, $F(8,187) = 1.14$, $p = .34$.

Model 3 did not explain more variance than Model 2, $F_{\text{change}}(2,184) < 1$. PDS and PDS x SAS remained significant, whereas neither FSSCR nor CDI contributed significantly to the prediction of AUCi, indicating that the relation is specific to social anxiety.

### 3.2 Social Anxiety and the Cortisol Response at Time 2

Model 1 was significant, $F(8,187) = 3.22$, $p = .002$, $R^2 = .121$. PDS showed a significant positive relation with AUCi, but the interaction with SAS was not significant. Model 2 explained more variance than Model 1, $R^2 = .135$, $F_{\text{change}}(1,186) = 2.92$, $p = .089$ at trend-level. A high cortisol concentration in the reference sample was associated with a larger AUCi. Importantly, the effect of PDS did not change, indicating that PDS was related to AUCi independent of possible effects on the reference concentration. An additional regression analysis, predicting the natural logarithm of the cortisol concentration in the reference sample from Model 1, $F(8,187) = 3.39$, $p = .001$, indicated that the reference concentration was positively related to PDS ($\beta = .23$, $p = .004$) at Time 2. It was also significantly related to female sex ($\beta = -.29$, $p < .001$) and use of oral contraceptives ($\beta = .18$, $p = .027$). Model 3 did not explain more variance than Model 2, $F_{\text{change}}(2,184) < 1$.

### 3.3 Results over time

Table 4 shows the regression weights of the explanatory variables in the regression analyses with clustered bootstrap (n = 196). Between-participant effects on the cortisol response in the analysis over time were comparable to the results at Time 1. AUCi was positively related to T1PDS, and the T1PDS x T1SAS interaction was significant. Among participants with low PDS-scores at Time 1
those with higher SAS-scores showed higher cortisol responses, but among participants with high
PDS-scores at Time 1 those with higher SAS-scores showed lower cortisol responses (see Figure 2).
Within-participants, the analysis showed a significant effect of pubertal development (ΔPDS) and
effects at the level of a trend for ΔSAS (90%CI [-0.27, -0.01]) and ΔSAS x T1PDS (90%CI [0.01, 0.27]). A
larger increase in pubertal development from Time 1 to Time 2 was associated with a larger cortisol
response, whereas a larger increase in social anxiety from Time 1 to Time 2 was associated with a
smaller cortisol response, at least in participants with lower levels of pubertal development at Time
1. Model 2 had a better fit to the data than Model 1, as indicated by a lower goodness of fit index,
QIC (Pan, 2001): Model 1 QIC = 770.3, Model 2 QIC = 768.4. The cortisol concentration in the
reference sample was positively related to AUCi at the trend-level. Importantly, the effects of
pubertal development and social anxiety did not change (ΔSAS 90%CI [-0.27, -0.01]; T1PDS x ΔSAS
90%CI [0.01, 0.28]), indicating that they were related to AUCi independent of possible effects on the
reference concentration. An additional regression analysis with clustered bootstrap, predicting the
natural logarithm of the cortisol concentration in the reference sample from Model 1, showed that
the concentration was significantly related to female sex (B = -0.19, 95%CI [-0.31, -0.07]), use of oral
contraceptives (B= 0.22, 95%CI [0.03, 0.41]), being tested alone (B = 0.11, 95%CI [0.02, 0.20]) and
ΔPDS (B = 0.08, 95%CI [0.01, 0.15], but not to social anxiety. Model 3 (QIC = 772.3) did not have a
better fit than Model 2.

4. Discussion

The present study investigated the relation between social anxiety and the cortisol response
to public speaking in adolescence, taking into account the effects of pubertal development on the
cortisol response. Because all variables were measured twice over a two-year interval, we were able
to examine both between and within-participant relations. Between participants, we found that
more socially anxious adolescents showed higher cortisol responses when their pubertal
development was low at Time 1, whereas they showed lower cortisol responses when their pubertal
development was advanced at Time 1. At Time 2, when most participants had reached higher levels
of pubertal development, the interaction disappeared and a negative main effect of social anxiety emerged, although it did not reach significance. Within-participants, an increase in social anxiety over time was associated with a lower cortisol response (particularly for participants with lower levels of pubertal development at Time 1).

The finding that the relation between social anxiety and the cortisol response to public speaking varied with the level of pubertal development (between-participants) may explain why no relation was found in previous studies that did not control for pubertal development (Martel et al., 1999; Schmidt et al., 1999; Miers et al., 2011; Kraemer et al., 2012). A previous study that specifically selected pre-pubertal children showed a positive relation between the cortisol response and social anxiety in childhood (Van West et al., 2008). In the present study, the relation between social anxiety and the cortisol response changed from positive at low levels of pubertal development to negative at high levels of pubertal development. Both findings are in line with the suggestion by Siess et al. (2014) that elevated responses in childhood turn into no difference and possibly attenuated responses in adolescence and adulthood.

A shift from elevated to attenuated responding can be explained by theories proposing that the stress response changes with the duration of the stressor, such as allostatic load theory (e.g. Fries et al., 2005) and PRISM (Tops et al., 2015; Tops et al., 2016). At the level of the HPA-axis, several changes may be involved (see Heim et al., 2000 for review). For example, in response to hypersecretion of Corticotrophin Release Factor (CRF) in the hypothalamus, CRF receptors in the pituitary may be down-regulated, resulting in a smaller release of adrenocorticotropic hormone (ACTH) by the pituitary and hence lower cortisol secretion by the adrenals. Furthermore, the negative feedback loop, by which cortisol suppresses the release of CRF and ACTH, may become more sensitive with prolonged stress.

These mechanisms may operate at any time of life in individuals. At a group level, however, we found evidence for a change from hyper- to hyporesponding in adolescence. Several developments may contribute to make adolescence the period in which the social challenges of
everyday life lead to an accumulation of allostatic load and activation of PRISM in socially anxious individuals. For example, forming more intimate relations with peers is an important developmental task for adolescents. They spend more time with peers and increasingly turn to peers instead of parents for support (Spear, 2000; Nelson et al., 2005). Social evaluation may become more consequential for adolescents in general and more threatening for some (e.g. those with negative self-views or poor social skills). Additionally, adolescence may be a biologically sensitive period (Gunnar et al., 2009; Del Giudice et al., 2011). Allostatic load may accumulate faster because puberty-related changes make the HPA-axis respond more strongly. (See Van den Bos et al. (2014) for a discussion of possible effects of increasing gonadal hormone levels on brain structures regulating the HPA-axis). Prospective research with socially anxious children is needed to investigate whether social-evaluative situations indeed become more threatening to them in adolescence and whether this results in accumulation of allostatic load and subsequent attenuation of the cortisol response.

The suggestion by Siess et al. (2014) that the relation between social anxiety and stress responses changes from positive in childhood to negative in adulthood implies that attenuated cortisol responses are expected in adults. Negative relations between social anxiety and cortisol have indeed been found in community samples of adults (Beaton et al., 2006; Shirotsuki et al., 2009). Results have been more varied in adults with clinical levels of social anxiety (i.e., SAD). As a group, they showed elevated cortisol responses (Condren et al., 2002; Roelofs et al., 2009) or no difference compared to control participants (Levin et al., 1993; Furlan et al., 2001; Klumbies et al., 2014). Detailed analyses, however, revealed lower cortisol responses in subgroups of participants with SAD (Furlan et al., 2001; Roelofs et al., 2009). The participants who showed lower cortisol responses seemed to be those who experienced more subjective distress.

Attenuated cortisol responses have also been related to other internalizing symptoms in adults. Yoon and Joormann (2012) suggested that hypocortisolism is specific for SAD with comorbid depression (though see Young et al., 2004). Other studies showed that the cortisol response to
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Public speaking correlated negatively with (general) trait anxiety (Jezova et al., 2004; Villada et al., 2016). In the present study, measures of depression and general anxiety were included as control variables. The results indicated that the cortisol response was specifically related to social anxiety. In a social-evaluative situation, social anxiety seems more relevant than other types of anxiety.

A limitation of the present study is the use of a post-task baseline taken 30 minutes after the public speaking task. Although the cortisol concentration in this sample was unrelated to social anxiety, it was positively related to pubertal development at Time 2, suggesting that more mature participants had not fully recovered yet. We dealt with this possibly confounding effect by statistically controlling the cortisol response to public speaking for the concentration in the reference sample (7). Effectivity of the LPST as a social-evaluative stressor was supported by the subjective nervousness ratings. The opportunity to prepare induced anticipatory stress rather than making the task less stressful. Nevertheless, replication with an impromptu public speaking task and in a real-life context would strengthen our results. In addition, although our findings are in line with theories proposing that lasting attenuation of the cortisol response results from an accumulation of allostatic load from the time of stressor onset (Fries et al., 2005; Del Giudice et al., 2011; Tops et al., 2016), the time of onset of heightened anxiety in social-evaluative situations was unknown in this study. Prospective studies are better suited to test these theories.

The present results highlight the importance of taking pubertal development into account in research on the cortisol response in adolescence. Among adolescents who had reached higher levels of pubertal development, those who were more socially anxious showed lower cortisol responses to public speaking. Attenuation of the cortisol response is typically considered the outcome of protective mechanisms (Fries et al., 2005; Del Giudice et al., 2011; Tops et al., 2016), because prolonged elevation of cortisol levels can cause tissue damage, which may disrupt biological systems (Miller et al., 2007). Yet, these protective mechanisms may contribute to maintaining social anxiety disorder. Reduced motivation to engage in social situations may result in avoidance or prevent socially anxious individuals from making an effort to be positively evaluated. This behavior may elicit
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less positive and more negative reactions from others and thus maintain anxiety (Rapee & Spence, 2004). A practical implication may be that treatment should offer alternative ways to limit the time spent in reactive control mode: by changing individuals’ interpretation of the situation as threatening and their (perceived) ability to respond to the challenge.
Acknowledgements

We thank Mark de Rooij for providing us with the R-script for regression analysis with clustered bootstrap and Anne Miers, Sindy Sumter, Laura Compier-de Block, Ellen Middag, Manja Koenders and Masha Takens for their assistance in data collection.

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Table 1. *Social Background Characteristics of Participants and Their Parents Assessed at Time 1.*

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country of birth participants (N = 314)</strong></td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>299</td>
</tr>
<tr>
<td>Thirteen different countries</td>
<td>15</td>
</tr>
<tr>
<td><strong>Country of birth parents (N = 455)</strong></td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>416</td>
</tr>
<tr>
<td>Twenty-two different countries</td>
<td>39</td>
</tr>
<tr>
<td><strong>Living situation (N = 321)</strong></td>
<td></td>
</tr>
<tr>
<td>With both biological parents</td>
<td>266</td>
</tr>
<tr>
<td>With biological mother only</td>
<td>19</td>
</tr>
<tr>
<td>With biological mother and stepfather</td>
<td>17</td>
</tr>
<tr>
<td>With each biological parent in alternation</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
</tr>
<tr>
<td><strong>Present education participants (N = 327)</strong></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>126</td>
</tr>
<tr>
<td>Prevocational secondary education</td>
<td>24</td>
</tr>
<tr>
<td>Senior general/ preuniversity secondary education (year 1)</td>
<td>38</td>
</tr>
<tr>
<td>Senior general secondary education (years 2-5)</td>
<td>66</td>
</tr>
<tr>
<td>Preuniversity secondary education (years 2-6)</td>
<td>73</td>
</tr>
<tr>
<td><strong>Highest completed education parents (N = 505)</strong></td>
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</tr>
<tr>
<td>Primary school or less</td>
<td>5</td>
</tr>
<tr>
<td>Prevocational education</td>
<td>88</td>
</tr>
<tr>
<td>Middle to higher secondary education</td>
<td>46</td>
</tr>
<tr>
<td>Middle vocational education</td>
<td>89</td>
</tr>
<tr>
<td>Higher vocational education</td>
<td>136</td>
</tr>
<tr>
<td>Note. In the Netherlands, senior general education is a five-year program preparing for higher vocational education. Preuniversity education is a six-year program preparing for university. Students are selected for either program during the first (combined) year.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Descriptives and Correlations

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>M</th>
<th>SD</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
<tr>
<td><strong>Time 1</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. PDS score</td>
<td>240</td>
<td>2.5</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. SAS score</td>
<td>246</td>
<td>2.3</td>
<td>0.7</td>
<td>-.060</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. FSSCR score</td>
<td>245</td>
<td>132.3</td>
<td>30.6</td>
<td>-.168**</td>
<td>.590***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. CDI score</td>
<td>245</td>
<td>9.0</td>
<td>5.3</td>
<td>-.096</td>
<td>.588***</td>
<td>.496***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Nervousness</td>
<td>245</td>
<td>62.9</td>
<td>27.2</td>
<td>-.194**</td>
<td>.366***</td>
<td>.356***</td>
<td>.291***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. AUCi</td>
<td>206</td>
<td>85.2</td>
<td>91.3</td>
<td>.431***</td>
<td>.000</td>
<td>.049</td>
<td>.004</td>
<td>.057</td>
<td></td>
</tr>
<tr>
<td>7. Reference</td>
<td>206</td>
<td>5.3</td>
<td>3.1</td>
<td>-.064</td>
<td>-.057</td>
<td>-.079</td>
<td>.021</td>
<td>.124†</td>
<td>.020</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. PDS score</td>
<td>247</td>
<td>3.1</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. SAS score</td>
<td>248</td>
<td>2.2</td>
<td>0.7</td>
<td>-.097</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. FSSCR score</td>
<td>248</td>
<td>124.7</td>
<td>30.5</td>
<td>-.071</td>
<td>.680***</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. CDI score</td>
<td>247</td>
<td>9.3</td>
<td>6.0</td>
<td>-.025</td>
<td>.450***</td>
<td>.343***</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Nervousness</td>
<td>240</td>
<td>53.0</td>
<td>28.2</td>
<td>-.200**</td>
<td>.291***</td>
<td>.301***</td>
<td>.121†</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6. AUCi</td>
<td>206</td>
<td>112.0</td>
<td>134.8</td>
<td>.156*</td>
<td>-.128†</td>
<td>-.153*</td>
<td>-.027</td>
<td>-.140*</td>
<td>-</td>
</tr>
<tr>
<td>7. Reference</td>
<td>206</td>
<td>6.5</td>
<td>3.9</td>
<td>.181**</td>
<td>-.054</td>
<td>-.184**</td>
<td>.051</td>
<td>.073</td>
<td>.166*</td>
</tr>
</tbody>
</table>

Note. PDS = Pubertal Development Scale (Petersen et al., 1988), SAS = Social Anxiety Scale for Adolescents (La Greca & Lopez, 1998), FSSCR = Fear Survey Schedule for Children Revised (Ollendick, 1983), CDI = Children’s Depression Inventory (Kovacs, 1992), Nervousness = rating of nervousness during the speech on a visual analog scale from 0 to 100, AUCi = Area Under the Curve
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with respect to increase (Pruessner et al., 2003). Increase was relative to the cortisol concentration in
the reference sample (i.e. sample 7).

†p < .10, *p < .05, **p < .01, ***p < .001
Table 3. Standardized regression weights for explanatory variables predicting the cortisol response at Time 1 and Time 2.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>PDS</td>
<td>.475***</td>
<td>.480**</td>
</tr>
<tr>
<td>SAS</td>
<td>.018</td>
<td>.021</td>
</tr>
<tr>
<td>PDS x SAS</td>
<td>-.150*</td>
<td>-.153*</td>
</tr>
<tr>
<td>Female</td>
<td>-.033</td>
<td>-.021</td>
</tr>
<tr>
<td>Medication</td>
<td>.081</td>
<td>.071</td>
</tr>
<tr>
<td>OC</td>
<td>-.001</td>
<td>-.012</td>
</tr>
<tr>
<td>Food intake</td>
<td>-.040</td>
<td>-.041</td>
</tr>
<tr>
<td>Tested alone</td>
<td>.078</td>
<td>.065</td>
</tr>
<tr>
<td>Reference sample</td>
<td>.108†</td>
<td>.113†</td>
</tr>
<tr>
<td>FSSCR</td>
<td>.083</td>
<td></td>
</tr>
<tr>
<td>CDI</td>
<td>-.041</td>
<td></td>
</tr>
</tbody>
</table>

Note. PDS = Pubertal Development Scale (Petersen et al., 1988), SAS = Social Anxiety Scale for Adolescents (La Greca & Lopez, 1998), OC = oral contraceptives, FSSCR = Fear Survey Schedule for Children Revised (Ollendick, 1983), CDI = Children’s Depression Inventory (Kovacs, 1992). Analyses were performed on the natural logarithm of the Area Under the Curve with respect to increase (Pruessner et al., 2003). Increase was relative to the cortisol concentration in the reference sample (i.e., sample 7).

†p < .10, *p < .05, **p < .01, ***p < .001
Table 4. *Unstandardized regression weights with 95% confidence intervals for explanatory variables of the cortisol response combined over Time 1 and Time 2.*

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>LL</td>
<td>UL</td>
</tr>
<tr>
<td>T1PDS</td>
<td>0.25</td>
<td>0.18</td>
<td>0.32</td>
</tr>
<tr>
<td>ΔPDS</td>
<td>0.28</td>
<td>0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>T1SAS</td>
<td>-0.03</td>
<td>-0.12</td>
<td>0.07</td>
</tr>
<tr>
<td>ΔSAS</td>
<td>-0.14</td>
<td>-0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>T1PDS x T1SAS</td>
<td>-0.11</td>
<td>-0.21</td>
<td>-0.02</td>
</tr>
<tr>
<td>T1PDS x ΔSAS</td>
<td>0.14</td>
<td>-0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>ΔPDS x T1SAS</td>
<td>-0.111</td>
<td>-0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>ΔPDS x ΔSAS</td>
<td>0.22</td>
<td>-0.05</td>
<td>0.50</td>
</tr>
<tr>
<td>Female</td>
<td>-0.06</td>
<td>-0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>Medication</td>
<td>0.16</td>
<td>-0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>OC</td>
<td>-0.25</td>
<td>-0.56</td>
<td>0.05</td>
</tr>
<tr>
<td>Food intake</td>
<td>-0.33</td>
<td>-0.65</td>
<td>-0.00</td>
</tr>
<tr>
<td>Tested alone</td>
<td>0.19</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>Reference sample</td>
<td>0.14</td>
<td>-0.01</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Note.* T1PDS = score on the Pubertal Development Scale (Petersen et al., 1988) at Time 1, ΔPDS = current PDS score minus PDS score at Time 1, T1SAS = score on the Social Anxiety Scale for Adolescents (La Greca & Lopez, 1998) at Time 1, ΔSAS = current SAS score minus SAS score at Time 1, OC = oral contraceptives, FSSCR = Fear Survey Schedule for Children Revised (Ollendick, 1983), CDI = score at Children’s Depression Inventory (Kovacs, 1992). The analyses were performed on the natural
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logarithm of the Area Under the Curve with respect to increase (Pruessner et al., 2003). Increase was relative to the cortisol concentration in the reference sample (i.e. sample 7). Bold print highlights that the 95% confidence interval did not include zero. Italic print indicates that zero was included in the 95% confidence interval, but not in the 90% confidence interval.
Figure 1. Mean cortisol concentration (nmol/l) in saliva samples taken directly before and 15, 25, 30, 35, 40 and 45 min after the beginning of the Leiden Public Speaking Task at Time 1 and Time 2. Asterisks indicate significant differences from the concentration in the reference sample (taken 45 min after the beginning of the task), based on Bonferroni-corrected pairwise comparisons.
Figure 2. Simple slopes plot illustrating the interaction effect of PDS and SAS on the predicted AUCi at Time 1. Lines represent the difference between the cortisol response associated with a SAS score of 1 SD below the mean and the cortisol response associated with a SAS score of 1 SD above the mean for an individual scoring 1 SD below the mean on PDS and for an individual scoring 1 SD above the mean on PDS. AUCi = Area under the curve with respect to increase (Pruessner et al., 2003). Increase was relative to the cortisol concentration in the reference sample (i.e. sample 7). The analyses were done on the natural logarithm of AUCi. PDS = Pubertal Development Scale (Petersen et al., 1988), SAS = Social Anxiety Scale – Adolescents (La Greca & Lopez, 1998).