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Adversity-driven changes in hypothalamic-pituitary-adrenal axis functioning during adolescence. The trails study

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ABSTRACT

The hypothalamic-pituitary-adrenal (HPA) axis has been proposed to be a key mechanism underlying the link between adversity and mental health, but longitudinal studies on adversity and HPA-axis functioning are scarce. Here, we studied adversity-driven changes in HPA-axis functioning during adolescence (N = 141). HPA-axis functioning (basal cortisol, cortisol awakening response, anticipation of, reaction to and recovery after a stress task) was measured twice, at age 16 and 19. Adversity (i.e., social defeat and loss/illness) since age 16 was measured extensively with the Life Stress Interview at age 19. Adolescents who reported being exposed to social defeat showed *increases* in basal cortisol (η^2 = 0.029) and *decreases* in reaction to the stress task (η^2 = 0.030) from age 16–19, compared to their peers in the loss/illness and no stress group. The current study provides unique longitudinal data on the role of adversity in HPA-axis functioning. Evidence is provided that adversity can affect the body's neuroendocrine response to stress, dependent on the nature of both the HPA-measures and adverse events under study.

Over the years, scientists have become increasingly interested in the biological mechanisms by which adversity "gets under the skin", and affects vulnerability and mental health (Miller et al., 2007). The hypothalamic-pituitary-adrenal (HPA) axis functioning has been proposed to be among the key biological mechanisms underlying the link between adversity and mental health (Burke et al., 2005; Herbert, 1997; Susman, 1998). A growing body of evidence shows that inter-individual variation in exposure to adverse events is related to inter-individual variation in HPA-axis functioning (Heim and Nemeroff, 2001; Kaufman et al., 2000; Sanchez, 2006). So far, much of the evidence is based on cross-sectional research, and studies vary with regard to the HPA-axis measures under study and the direction of the effects found. For example, with regard to basal morning cortisol, elevated as well as lower (blunted) levels have been found in individuals exposed to adverse events (Bruce et al., 2009; Cicchetti and Rogosch, 2001; Heim et al., 2000; Miller et al., 2007; Trickett et al., 2010). Also concerning cortisol responses to (laboratory) stress both increased (Heim et al., 2000; Tyrka et al., 2008), and blunted responses were found (Elzinga et al., 2008; MacMillan et al., 2009; Ouellet-Morin et al., 2011; Peckins et al., 2012).

Although findings from cross-sectional research are highly inconsistent, some patterns seem to emerge when the nature and timing of the adversity are taken into account. First, and with regard to the nature of the adverse events, in a recent study by Kuhlman et al. (2015), it was demonstrated that different types of childhood adversity were differentially related to HPA-axis functioning during adolescence (Kuhlman et al., 2015). Whereas emotional and physical abuse were related to hyper-reactivity (faster reactivity and slower recovery) in stress-induced cortisol levels, non-intentional adversity such as witnessing an accident did not affect acute stress reactivity but was related to elevated cortisol at bedtime. Second, and with regard to the timing and duration of the adverse events, research suggests that the HPA-axis responds to acute stress with (sustained periods of) increased cortisol secretion (*hyper-reactivity*). Only if the adverse experiences are particularly lasting, the HPA-axis may show down-regulating of cortisol secretion (*hypo-reactivity)* (MacMillan et al., 2009; Miller et al., 2007). Consistent with this pattern is the "attenuation hypothesis", positing that persistent activation of the HPA-axis eventually downgrades stress reactivity to limit physiological, emotional and behavioural responses to stress (Trickett et al., 2010; Susman, 2006). Whereas this attenuation may be temporally adaptive in a demanding or changing environment, lasting attenuation of the stress system has been considered to play a crucial part in adverse life outcomes such as persistent antisocial behavior (Susman, 2006).

Although the attenuation hypothesis is plausible given the findings

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mentioned before, only a few studies have examined HPA-axis functioning using a longitudinal design. Doom et al. (2014) demonstrated that maltreated and non-maltreated children differed in longitudinal (20-weeks) cortisol patterns. Specifically, maltreated children showed higher variance in the initial cortisol levels and slope over time, and maltreated children with higher cortisol at the first assessment showed cortisol suppression over time, indicating potential attenuation of the HPA-axis after chronic high cortisol levels. Trickett et al. (2010) found that, whereas increases in basal cortisol levels across development were normative, victims of childhood sexual abuse had somewhat higher basal cortisol levels shortly after disclosure of being abused, but showed smaller increases over time than non-abused children. Finally, two studies provided support for the attenuation hypothesis in the context of stress-induced HPA-axis activity (Peckins et al., 2012, 2015). Peckins et al. (2012) found that exposure to violence was related to blunted responses to stress in children with no identified serious mental health problems or reports of maltreatment. In their 2015 study, stress-induced HPA-axis activity was measured three times with circa 2-year intervals between waves (Peckins et al., 2015). Results showed that maltreated children were more likely than non-maltreated children to show blunted cortisol reactivity at the first and second, but not the third wave, even after controlling for recent exposure to adversity.

Together, findings suggest a link between exposure to adverse events and HPA-axis functioning. However, much remains unknown with regard to the nature of the associations (Jaffee et al., 2015). The direction of the effects, however, seems to vary as a function of the nature and timing/duration of the adverse events, but longitudinal studies are scarce and no studies seem to have focussed on adversity-driven changes in both basal and stress-induced HPA-axis functioning. As such, in the current study we examined whether and how adolescent adversity is related to changes in HPA-axis functioning. As the role of adversity in HPA-axis functioning is likely to differ dependent on the measures under study, a range of measures was taken into account. These include the traditionally studied basal cortisol and reaction to a social stress task (Ouellet-Morin et al., 2011; Tarullo and Gunnar, 2006) as well as three less used indicators of HPA-axis functioning: the cortisol awakening response (CAR), anticipation of and recovery after the stress task (Nederhof et al., 2015). *CAR* can be considered an indicator of anticipation of the demands of the upcoming day (Hellhammer et al., 2007). *Anticipation stress* reflects unpredictability of the environment and reduced *recovery* afterwards is an indicator of lack of control over the situation (Koolhaas et al., 2011).

In addition, we will examine different types of adversity: 1) *social defeat*, including events that have repeatedly been shown to be linked to HPA-axis functioning like being a victim of abuse, is known to have a profound impact and to be often (partly) intentional, and 2) *loss/illness*, including events that also have a profound impact, but are generally non-intentional, i.e., death and severe illness of a loved one. It is hypothesized that adversity is related to changes in HPA-axis functioning. We assume that a certain level of uncontrollability and unpredictability is conditional for adversity to have the potential to affect the HPA-axis (Fries et al., 2009), which may be the case for both social defeat and loss/illness. However, based on the existing literature, effects are expected to be larger for social defeat than for loss/illness. Whether the HPA-axis will respond to adversity with hyper-reactivity (Kuhlman et al., 2015) or hypo-reactivity (e.g., Peckins et al., 2015) will be explored.

1. Methods

1.1. Sample

The current study used subsample data of the Tracking Adolescents' Individual Lives Survey (TRAILS) participants, collected at the third and fourth wave. This subsample consisted of participants who 1) agreed to participate in the laboratory part of the third wave (mean age = 16.1, SD = 0.59); 2) were assessed with the Life Stress Interview LSI (Kendler et al., 1998) at the fourth wave (mean age = 19.1, SD = 0.68); 3) were, if LSI was rated a 3 or 4 on the severity and person-independency scales by two independent researchers/raters, assigned to either a social loss group (i.e., if they had been exposed to death and serious illness in their close environment such as family, or a close friends), social defeat group (i.e., if they had been a victim of bullying, sexual intimidation or violence or were dumped after a serious relationship), or comparison group (i.e., randomly selected from the pool of participants that experienced no loss or defeat, or a loss or defeat that was rated 1 or 2 on the severity and/or and person-independency scale); and 4) agreed to participate in reassessments of laboratory tasks at the fourth wave.

Compared to the rest of the TRAILS sample, adolescents with at least one risk factor for mental health problems had a slightly greater chance of being selected for the laboratory tasks at age 16. Nevertheless, the subsample did not differ on any of the risk factors (see for more details: Bouma et al., 2009). In total, 178 adolescents participated in the behavioural experiments both at age 16 and 19 and in the LSI at age 19 (no stress = 68; social defeat = 32 and loss/illness = 61, 17 participants experienced both social defeat and loss/illness and were included in both adversity groups). For one participant from the loss/defeat and social defeat group we did not have any data, resulting in a sample of 177 adolescents for the current study (46.3% girls). Due to some missing failed saliva analyses the sample varied slightly across analyses. All individuals provided informed consent or assent, and the study was approved by the UMCG Research Ethics Committee. For a detailed description of the sample selection, procedures, methods, and attrition rates, see Nederhof et al. (2012) and Laceulle et al. (2015).

1.2. Social stress test

This test was the last challenge of the experimental sessions at age 16 and 19. It involved a standardized protocol including public speaking and mental arithmetic, inspired by the Trier Social Stress Task (Kirschbaum et al., 1993), for the induction of moderate performance-related social stress. At age 16, the participants were instructed to prepare a 6-min speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged by a panel of peers after the experiment. The participants had to speak continuously for the whole period of 6 min. The speech was followed by a 3-min interlude in which the participants were not allowed to speak. After the interlude, participants were instructed to subtract 17 repeatedly, starting with 13.278. This difficult task was meant to induce a sense of uncontrollability. At age 19, the test protocol was identical to the protocol at age 16 except two differences: 1) Adolescents were asked to present in front of a committee instead of a camera. Participants were told that the committee judged content of the presentation as well as gesture. 2) Instead of presenting about themselves and their lives, adolescents were instructed to convince the committee to either hire them for a job or select them for an educational program.

1.3. HPA-axis functioning

1.3.1. Basal cortisol and cortisol awakening response

To collect data on basal cortisol and CAR, both at age 16 and at age 19 participants received a verbal and written instruction to collect saliva at home immediately after waking up as they were still lying in bed (CM1) and 30 min after awakening (CM2), using the Sarstedt Salivette device (Nümbrecht, Germany). Directly after sampling, saliva samples were stored by participants in their refrigerator and brought to the institute as soon as possible.

1.3.2. Stress-induced cortisol

We assessed HPA-axis responses towards the GSST using four

cortisol samples (referred to as CE1, CE2, CE3, CE4). There is a delay of approximately 20 min between the production of cortisol by the adrenal glands and the detectability of representative levels of cortisol in saliva. CE1, reflecting anticipatory cortisol levels, was taken at the start of the experimental session. CE2 was collected just before the GSST, reflects HPA axis activity approximately 20 min earlier, and is considered a pre-test measure. CE3 was collected directly after the end of the GSST and reflects cortisol levels during speech. CE4, collected 40 min after the end of the GSST reflects post-test cortisol level.

1.3.3. Other variables

Previous research have suggested a range of parameters which may affect cortisol measures or the link between adversity and HPA-axis functioning. These include, but are probably not limited to, sex, date in menstrual cycle, use of oral contraceptives and medication, depressive symptoms, smoking, alcohol and substance use, BMI, exercise and eating prior to the experiment and SES (Bouma et al., 2009; Ginty et al., 2014; Herbison et al., 2016; Jones et al., 2013; Morris et al., 2016; Obasi et al., 2015; Schorr et al., 2015). Based on these earlier findings and the data available, it was examined which variables were associated with the cortisol measures and stress groups. Variables that showed a significant link were then adjusted for accordingly in the regression analyses (see below). In short, medication use and sex were related to CAR (respectively, $t_{medication}$ (174) = 1.85, $p = 0.046$ and t_{sex} (174) = 1.98, $p = 0.049$), sex and use of oral contraceptives were related to reaction to the stress task (respectively, t_{sex} (171) = −6.10, $p < 0.001$ and $t_{contraceptives}$ (171) = 3.37, $p = 0.001$) and use of oral contraceptives was related to anticipation to stress ($t_{contraceptives}$ (174) = −2.19, $p = 0.030$). Also, depressive symptoms were related to reaction to stress (but not to any of the other cortisol measures): $r = 0.16$, $p = 0.038$. Exercise prior to the experiment, smoking, substance use and SES were not related to either the cortisol measures or the stress groups. Full statistics are available in Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.psyneuen. Thus, sex, medication use, use of oral contraceptives and depressive symptoms were included as potential confounding variables in the regression analyses.

1.4. Statistical analyses

Analyses were performed in SPSS (Version 22.0). We first calculated descriptive statistics of the (untransformed) variables used in this study, split for the three adversity groups. All cortisol variables were transformed and standardized to have a mean of 0 and a standard deviation of 1 before further analysis. The standardized score of CM1, the first cortisol measure directly after awakening, was used as a measure of basal cortisol. The cortisol awakening response (CAR) was calculated as CM2-CM1 and then standardized. The standardized score of CE1 was used as a measure of anticipation to stress. Reaction and recovery were calculated by saving the standardized residuals from regression analyses: 1) for reaction, stress task cortisol (speech, CE3) was predicted by the pre-test measure (CE2); 2) for recovery, post-test cortisol (CE4) was predicted by the task measure (CE3). Standardized residuals are the residuals divided by an estimate of their standard deviation and have, similar to normal z-scores, a mean of 0 and a standard deviation of 1. Scores reflect the distance to the regression line and can consequently be used as a measure of individual differences in change during the experiments. Bivariate correlations based on the transformed cortisol measures were then added to the descriptive statistics. Associations between adversity and HPA-axis functioning were examined using Univariate ANOVA's, one for each of the cortisol measures. Dependent variables were the HPA-axis functioning measures at T4. Independent variables were the HPA-axis functioning measures at T3 and the two adversity dummies (i.e., social defeat (1) vs the rest (0) and social loss (1) vs the rest (0)).

Table 1
Descriptive characteristics for the untransformed cortisol variables.

	No Adversity		Social Loss		Social Defeat	
	M	*SD*	M	*SD*	M	*SD*
Age 16						
CM1	7.96	4.11	8.73	5.46	8.55	4.29
CM2	13.16	6.19	13.70	5.87	13.69	5.41
Pre-experiments (CE1)	4.73	3.47	5.40	5.62	4.19	2.45
Pre-stress task (CE2)	3.18	1.93	4.10	4.89	3.12	1.52
Stress (speech) (CE3)	4.51	2.39	5.23	4.37	4.40	3.42
Post-stress task (CE4)	3.54	2.00	4.41	6.65	3.55	2.45
Age 19						
CM1	8.61	5.04	8.67	5.00	10.18	5.21
CM2	15.49	7.32	15.69	7.11	16.16	7.97
Pre-experiments (CE1)	6.11	3.97	6.38	3.97	5.99	2.56
Pre-stress task (CE2)	4.59	2.97	5.25	4.37	4.51	1.77
Stress (speech) (CE3)	8.19	4.90	7.98	6.02	6.57	4.22
Post-stress task (CE4)	5.52	3.04	5.63	3.32	4.99	2.99

Note: Descriptives for cortisol data (in nmol/l) reflect untransformed data.

2. Results

Descriptive statistics of the untransformed cortisol variables, at age 16 and 19 and split for the three adversity groups, are reported in Table 1. Correlations between all main study variables are reported in Table 2. Stability over time (i.e., from age 16 to 19) for the cortisol measures are presented in bold. Stability over time was low for all cortisol measures, except for reaction to stress for which stability was moderate (basal cortisol $r = 0.12$, $p = 0.131$; CAR $r = -0.04$, $p = 0.678$; anticipation $r = 0.20$, $p = 0.034$; reaction $r = 0.28$, $p = 0.001$; recovery $r = 0.09$, $p = 0.280$). In addition to the correlations for the total group, stability over time was examined for adolescents in the no stress group to get an indication of normative change. Here, stability over time was low for all cortisol measures except for anticipation and reaction to the stress task: Basal cortisol $r = 0.20$, $p = 0.128$; CAR $r = -0.06$, $p = 0.665$; anticipation $r = 0.45$, $p = 0.004$; reaction $r = 0.36$, $p = 0.008$; recovery $r = 0.06$, $p = 0.647$ (Fig. 1).

Associations between adversity and subsequent HPA-axis functioning were examined using univariate ANOVAS's, while controlling for initial cortisol levels, sex, medication use, use of oral contraceptives and depressive symptoms. Model estimates for the effects of initial cortisol levels and adversity are reported in Table 3. In line with the low stability over time, cortisol responses at age 16 did not significantly predict cortisol responses at age 19, except for reaction to stress. Adolescents who −compared to their peers- showed a strong response at age 16, also showed a strong response to the stress task at age 19 (B = 0.27, SE = 0.08, $p = 0.001$, $\eta^2 = 0.079$). With regard to the effect of adversity, social defeat was related to changes in both basal cortisol and reaction to stress during adolescence. Specifically, adolescents who reported being exposed to social defeat showed *higher* basal cortisol (B = −0.39, SE = 0.19, $p = 0.040$, $\eta^2 = 0.029$) and *lower* reaction to the stress task (B = 0.33, SE = 0.17, $p = 0.048$, $\eta^2 = 0.030$). No effects were found for CAR, anticipation to stress and recovery after stress. Exposure to loss/illness was not related to any of the cortisol variables at age 19.

3. Discussion

The current study provides unique longitudinal data on the association between adversity and changes in HPA-axis functioning during adolescence. HPA-axis activity (basal cortisol, cortisol awakening response, anticipation and reaction to and recovery after a stress task) was measured twice, at age 16 and 19. With regard to adverse events a distinction was made between social defeat (i.e., violence and severe

Table 2
Bivariate correlations between all main study variables.

	1	2	3	4	5	6	7	8	9	10	11
1. Basal cortisol (16)											
2. CAR (16)	−0.41*										
3. Anticipation (16)	0.32*	−0.28*									
4. Reaction (16)	0.18	−0.03	0.02								
5. Recovery (16)	0.23*	−0.16	0.61*	−0.44*							
6. Basal cortisol (19)	**0.12**	0.00	−0.08	−0.01	−0.11						
7. CAR (19)	0.01	**−0.04**	0.13	0.18*	−0.02	−0.25*					
8. Anticipation (19)	0.02	0.12	**0.20***	0.18*	−0.06	−0.14	0.11				
9. Reaction (19)	0.14	−0.06	−0.01	**0.28***	−0.26*	−0.01	.26*	0.06			
10. Recovery (19)	−0.04	−0.10	0.04	0.05	**0.09**	0.02	0.05	.33*	−0.18*		
11. Social Defeat	−0.02	0.00	−0.06	0.06	−0.03	0.14	−0.08	−0.05	−0.17*	04	
12. Social Loss	0.06	0.02	0.13	−0.04	0.02	−0.06	0.03	0.03	−0.02	0.09	−0.13

Note: $^{*}p < 0.05$. Values in **bold** reflect stability over time for the cortisol measures.

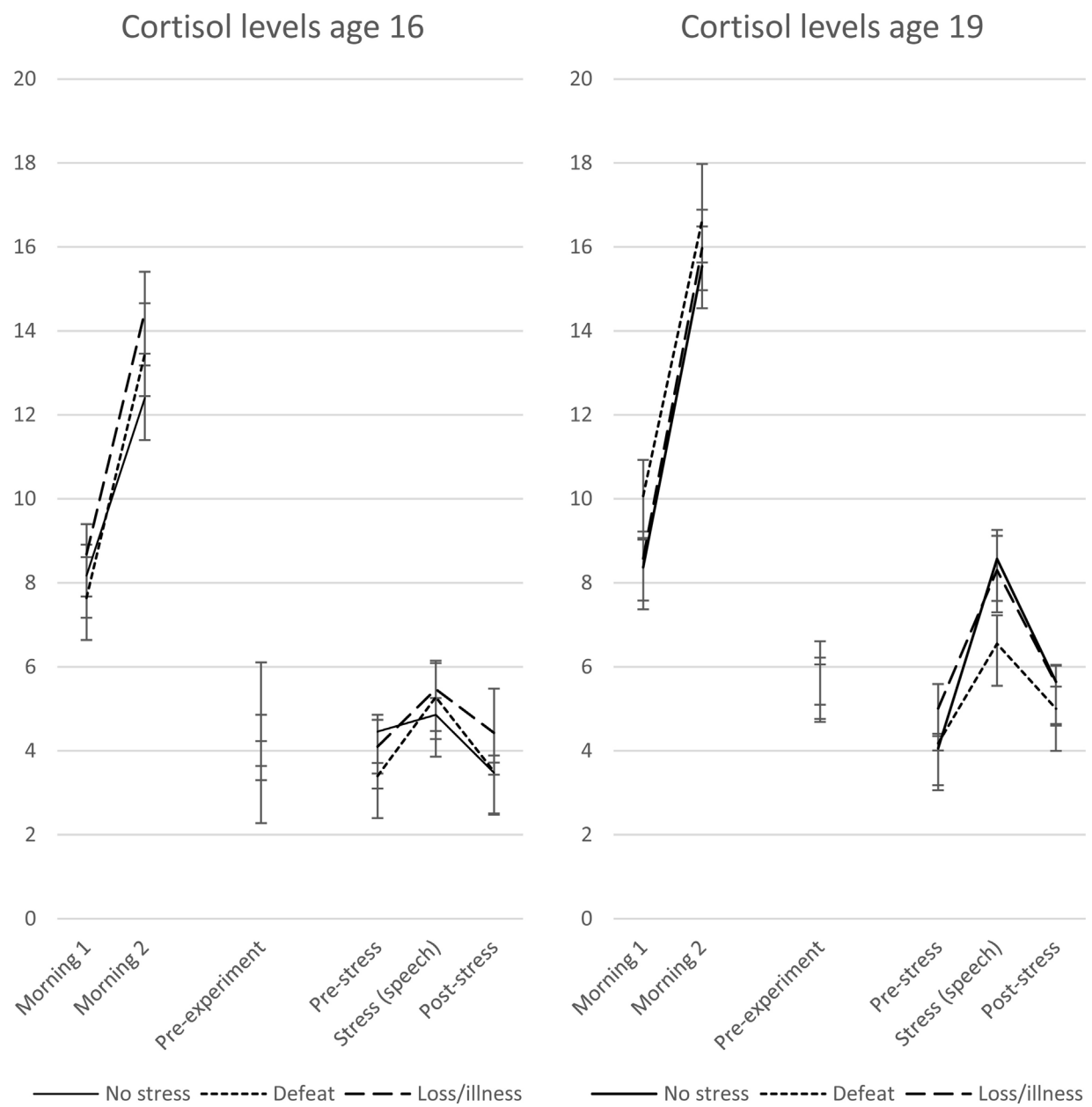

Fig. 1. Graphic representations of the various cortisol measures, split for the three adversity groups, at age 16 and at age 19. The cortisol measures are the untransformed variables as presented in Table 1. Cortisol values are in nmol/l.

Table 3
Associations between adversity and the cortisol variables (between-subject effects).

	F	df(error)	p	η^2	R^2
Basal cortisol (age 19)					0.066
Basal cortisol (16)	1.95	1(146)	0.165	0.013	
Social defeat (16–19)	**4.30**	**1(146)**	**0.040**	**0.029**	
Social loss/illness (16–19)	0.20	1(146)	0.655	0.001	
CAR (age 19)					0.055
CAR (16)	0.05	1(108)	0.828	0.000	
Social defeat (16–19)	0.00	1(108)	0.999	0.000	
Social loss/illness (16–19)	0.1	1(108)	0.753	0.001	
Anticipation (age 19)					0.071
Anticipation cortisol (16)	1.34	1(108)	0.250	0.012	
Social defeat (16–19)	0.12	1(108)	0.726	0.001	
Social loss/illness (16–19)	0.07	1(108)	0.797	0.001	
Reaction cortisol (age 19)					0.245
Reaction cortisol (16)	**10.85**	**1(127)**	**0.001**	**0.079**	
Social defeat (16–19)	**3.98**	**1(127)**	**0.048**	**0.30**	
Social loss/illness (16–19)	0.20	1(127)	0.657	0.002	
Recovery (age 19)					0.074
Recovery cortisol (16)	0.90	1(131)	0.343	0.007	
Social defeat (16–19)	1.42	1(131)	0.236	0.011	
Social loss/illness (16–19)	2.52	1(131)	0.115	0.019	

Note: All analyses were controlled for sex, use of oral contraceptives, medication and depressive symptoms.
Bold is $p < 0.05$.

bullying), and loss and severe illness of loved ones. Adolescents who reported being exposed to social defeat (but not loss/illness) showed *increases* in basal cortisol and *decreases* in reaction to the stress task from age 16 to 19, compared to their peers. Adversity was not related to CAR, anticipation to stress or recovery after stress.

Despite the growing support for associations between inter-individual variation in exposure to adverse events and inter-individual variation in HPA-axis functioning (Heim and Nemeroff, 2001; Kaufman et al., 2000; Sanchez, 2006), there has been ongoing debate regarding the nature of such links (Jaffee et al., 2015). Nonetheless, from the broader literature it appears that acute, short-term stress exposure is related to increased HPA-axis functioning. Only when cortisol levels remain consistently high, without sufficient recovery in between (adverse) events, the body responds with down-regulation of the stress-system to prevent the damaging effects of continuously high basal cortisol on the brain (De Bellis and Kuchibhatla, 2006).

Our findings on basal cortisol seem to provide support for the first half of the mechanism (up-regulation). Possibly, basal cortisol has a delayed response to adversity, and once affected initially continues with up-regulation (i.e., increased cortisol secretion). Only when adverse experiences are especially severe and lasting, basal cortisol may responds to adversity with attenuation. This would be in line with the suggestion that basal cortisol may be a relatively stable, trait-like component of HPA-axis functioning (Hellhammer et al., 2007), and as such may be less easily affected by environmental influences than more state-like aspects of HPA-axis functioning such as reactivity to an acute stressor. Nonetheless, future research is needed disentangling the state- and trait component of the various cortisol measures before any conclusions can be drawn on this issue.

In contrast, the findings on reaction to stress provide support for down-regulation of the HPA-axis. Following the line of reasoning above, adversity may affect state-like components (i.e., reaction to acute stress) of HPA-axis before affecting more trait-like components (i.e., basal cortisol), resulting in decreased reactivity to our social stress task. Thus, the social defeat experiences captured in the current study may be sufficiently severe and lasting to affect reactivity to acute stress by down-regulation of the HPA-axis, but not severe or lasting enough to result in down-regulation of basal cortisol levels. The findings on social defeat and reaction to stress would also explain why studies on sexual abuse and maltreatment (like social defeat relatively severe and/or lasting experiences) seem to show some trends towards blunted cortisol responses to acute stress as measured with a stress task (MacMillan et al., 2009; Ouellet-Morin et al., 2011; Peckins et al., 2015).

However, given the mostly inconsistent findings in previous research on adversity and HPA-axis functioning and ongoing lack of longitudinal research, over-interpretation of our results needs to be avoided. Our findings on basal cortisol and reaction to stress, as well as the non-significant findings for CAR, anticipation and recovery, primarily demonstrate that indicators of HPA-axis functioning are not all equally affected by adverse experiences during adolescence. Nor do all types of adversity have the same potential to alter HPA-axis functioning. As such, several questions remain.

First, following the theoretical model by Koolhaas et al. (2011), we hypothesized that in addition to reaction to stress, also anticipation to and recovery after the social stress task may be affected by adverse experiences. We did not find support for such effects. This may be particularly surprising with regard to recovery after stress, given the delayed recovery after acute stress in maltreated youth reported by Kuhlman et al. (2015). However, this difference might well be due to the cross-sectional nature of the study by Kulhman et al. When zooming in on our descriptive statistics for age 19 only (without controlling for recovery at age 16), results seem to suggest a similar trend of lower recovery after acute stress in adolescents exposed to social defeat in the preceding years (ΔCE4-CE3$_{defeat}$ = 0.55 vs. ΔCE4-CE3$_{nostress}$ = 2.94, see Table 1).

Second, exposure to loss or illness did not affect HPA-axis functioning. The stronger effect of social defeat is in line with our hypothesis and earlier studies providing strongest and most consistent support for a link between victimization (sexual, physical and emotional) and HPA-axis functioning. A theoretical rational why particularly these events may affect cortisol responses may be that social defeat is often characterized by (partly) intentional adversity. Intentional adversity may be experienced as a threat to the physical or emotional integrity (MacMillan et al., 2009; Ouellet-Morin et al., 2011), which is less likely for non-intention adversity such as loss of a loved one. Nonetheless, replication is crucial and future research should make an effort to recruit samples sufficiently large and diverse to enable differentiation between multiple types of adverse events.

Third, both the longitudinal studies on basal cortisol by Doom et al. (2014) and Trickett et al. (2010) and the study on reactivity by Peckins et al. (2015) suggest that effects of adversity on changes in HPA-axis functioning vary as a function of time since exposure or disclosure. Although the prospective nature of the current study is clearly one of its strengths, more waves and possibly also smaller time gaps are needed to look at more subtle patterns such as the role of when the event took place, duration of the event and persistence of the effects (permanent scarring of HPA-axis functioning vs. a temporarily dip).

Fourth, whereas the current design allows a comparison between stress groups, the design does not allow to make statements on normative changes in HPA-axis functioning (Gunnar et al., 2009). Change in the no stress group may seem to provide some indication of normative change, but caution is needed in interpreting these results as there was a small change in the design of the stress task from age 16 to age 19. That is, whereas adolescents presented in front of a camera at age 16, they presented in front of a committee at age 19. As such, the tasks are not fully comparable. However, the findings do provide some interesting information on stability over time (i.e., changes in the relative placement of individuals compared to their peers).

Stability from age 16 to 19 (in the no-stress group) was low for basal cortisol, CAR and recovery after stress, but quite substantial for anticipation and reaction to stress. The low stability might be particularly surprising with regard to basal cortisol, which has been suggested to be a relatively stable, trait-like characteristic (Hellhammer et al., 2007). However, measurement error may have been relatively large for basal cortisol (and CAR) as these were sampled at the participant's home,

without the presence of a test-assistant. Adolescents may not have followed the written instructions strictly, resulting in limited resemblance between the settings at age 16 and 19, and subsequent low stability over time. A related issue is that it is increasingly emphasized that at least three samples are required to accurately measure CAR (Stalder et al., 2016). If there are only two samples collected it is uncertain if the second sample is part of a peak or recovery in cortisol changes post awakening. As such, thirty minutes post awakening may not capture the peak or the trajectory towards recovery, which may have further increased the error in our CAR measure. With regard to stress-induced cortisol, although the design of the social stress task at age 19 was slightly different from the task at age 16, the setting was highly standardized which may explain the relatively high stability of reaction to stress from age 16 to 19.

To conclude, the current study extends previous research on how adversity "gets under the skin" and affects HPA-axis functioning during adolescence. The findings on (increased) basal cortisol and (decreased) reaction to stress after social defeat, as well as the non-significant findings for CAR, anticipation and recovery, emphasize that various indicators of HPA-axis functioning are not equally affected by adverse experiences during adolescence. Similarly, the potential of adverse events to alter HPA-axis functioning may depend on the nature, duration and time with respect to the cortisol assessment. This is an important finding contributing to previous research because it differentiates between indicators of HPA-axis functioning and between different types of adverse experiences. Nonetheless, more longitudinal research is needed to further improve our understanding of how adversity can affect the body's neuroendocrine response to stress, and the way by which the link between adversity and HPA-axis functioning varies as a function of both the HPA-measures and adverse events under study.

Conflict of interest

All authors declare that they have no conflict of interest.

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