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A B S T R A C T

Objective: Previous research has identified a link between peer victimization and depressive symptoms during adolescence. The goal of the current study is to examine the possible indirect effects of HPA axis dysregulation in the link between adolescent peer victimization and depressive symptoms.

Method: A total of 113 boys (*n* = 61) and girls (*n* = 52) participants from grade 5 (*M* age = 10.31 years) and grade 6 (*M* age = 11.33 years) who were predominantly European-Canadian completed self-report measures of peer victimization and depressed affect as well as, measures of salivary cortisol and self-reports of negative experiences collected in conjunction with one another five times per day over the course of four school days.

Results: Multi-level structural equation modeling found that peer

victimization was indirectly associated with depressive symptoms via blunted cortisol reactivity (i.e. increases in cortisol in response to a negative event) but only at high rates of chronic peer victimization.

Conclusion: The findings suggest that future studies should focus on HPA axis dysregulation for better understand the development, as well as the stability of depression over time and that interventions targeting peer victimization may want to put a special focus on those who are chronically experiencing peer victimization over time.

Bi-directional associations have been observed between depressive symptoms and peer victimization (see the following meta-analyses: Hawker and Boulton, 2000; Reijntjes et al., 2010). Peer victimization is known to predict increases in depressive symptoms over time just as depressive symptoms have been observed to predict increases in peer victimization over time (Reijntjes et al., 2010). The inter-relation be- tween peer victimization and depressive symptoms found during adolescence persists beyond this specific developmental time period of adolescence. For instance, peer victimization during early and middle adolescence has been found to predict depressive symptoms and incidence of major depressive disorder into adulthood, even after control- ling for earlier rates of depressive symptoms during adolescence (Copeland et al., 2013; Takizawa et al., 2014), indicating that the effects of peer victimization in adolescence on depression reaches into adulthood.

While it is clear that there is a link between peer victimization and depressive symptoms during adolescence and that the effect of peer victimization in adolescence on depressive symptoms is persistent over time, the mechanism by which peer victimization and depressive symptoms are connected in a reciprocal manner is maintained over time is not clear. This information is critical toward understanding and, possibly addressing, the long-term impact of peer victimization, and other negative peer experience, on depression as suggested by others (Vaillancourt et al., 2013; Wichers et al., 2010). The goal of the current study is to provide evidence of the HPA axis as a possible mechanism of the association between peer victimization and depressed affect by measuring HPA functioning with salivary cortisol levels and measuring reports of negative experiences in conjunction to one another multiple times a day across multiple days.

Activity of the HPA provides a likely mechanism to explain the as-sociation between peer victimization since it functions as a secondary response system to stressful experiences, such as peer victimization, secreting cortisol in response to these experiences and affecting the metabolic, cardiovascular, and memory systems to respond to the stress. Additionally, stressful experiences have

been shown to be directly linked to increases in cortisol, especially if they are uncontrollable and/or social-evaluative in nature (Dickerson and Kemeny, 2004), and importantly in terms of serving as a mechanism, over time the exposure to negative, stressful experiences has also been shown to alter the operation of this system leading to dysregulation as it becomes hyper- or hypo-reactive to stressful experiences depending on various factors such as the proximity to the event or experiences and such dysregulation can become stable over time (Cicchetti and Rogosch, 2001; Miller et al., 2007; Trickett et al., 2010) and it has been suggested that this stably, dysregulated HPA axis could help to explain the stability of major depressive disorder (Menke, 2019; Guerry and Hastings, 2011; Wichers et al., 2010). Some have suggested that this specific type of dysregulation occurs when the glucocorticoid receptors become less sensitive leading to a reduced feedback loop possibly as a result of the HPA system switching from being regulated by corticotropin-releasing hormone to arginine vasopressin (Menke, 2019; O'Keane et al., 2012). In respect to the current study, chronic peer victimization may serve to impact the operation of the HPA axis and result in its dysregulation in general and in how it responds to stressful experiences.

The current study examines two patterns of dysregulation of the HPA axis as a possible mechanism: cortisol diurnal daily patterns and cortisol reactivity patterns. The typical daily cortisol pattern in individuals has the highest rates of cortisol for the day just after waking with a gradual curvilinear decrease in cortisol levels over the course of the day. Since the measurement of cortisol is highly dependent on the time of day that the measurement is taken, measuring cortisol at one time point in the morning or one time point in the afternoon is not ideal for understanding the HPA axis functioning. In terms of diurnal daily patterns, a dysregulated HPA axis would be indexed in terms of specific patterns of in the diurnal curve of the secretion of cortisol over the course of the day. In this respect, a dysregulated diurnal pattern refers to a flatter (i.e., hypo- reactive) or steeper (i.e., hyper-reactive) slope of cortisol levels over the course of the day. The current study also examined HPA axis dysregulation by directly measuring changes in cortisol levels in response to stressful events, in what we

call reactivity. Given that one of the main functions of the HPA axis is to react to stressful events, we wanted to test reactivity dysregulation of the HPA axis to stress in terms of small blunted increases in cortisol levels (i.e. hypo-reactive) and large, spiked increases (i.e. hyper-reactive) in response to stressful events. To be able to create a measure of reactivity, we utilized a successful approach from a previous study (Adams et al., 2011). First, we modeled each individual participant's daily cortisol pattern by using multiple measures of cortisol over the course of a school day for multiple days. This method is advantageous as it provides a reliable baseline index across each time point of the school day for each participant. Next, we measure the amount of change in cortisol levels against the baseline cortisol measurement at each time point that occurs in reaction to reports of the level of negativity of the experience at that time point. In other words, this measure of cortisol reactivity is the average amount of that cortisol increases from the modeled baseline measure of cortisol at each time point as predicted by the level of negativity of the experience measured at each time point.

To provide initial evidence of the HPA axis functioning (i.e., cortisol diurnal daily patterns and cortisol reactivity) serving as a mechanism of the association between peer victimization and depression, the current study tested the indirect effect of HPA axis functioning on this association. For an indirect effect to be present, the predictor must be associated with the indirect variable and the indirect variable must be associated with the outcome measure. Although no study has directly tested the indirect effects of cortisol patterns of the link between peer victimization and depressive symptoms, findings from previous studies of cortisol diurnal and reactivity patterns support testing such indirect effects, as reviewed below. These studies provide evidence of the separate components of an indirect pathway: the association between peer victimization and HPA dysregulation (i.e. evidence of the association between the predictor variable and indirect variable) and the association between HPA dysregulation and depression and depressive symptoms (i.e. evidence of the association between the indirect variable and the outcome variable).

Peer Victimization and Cortisol/HPA Axis Dysregulation

Multiple studies conducted with adolescents provide evidence of the association between our hypothesized predictor and indirect variables, specifically between being a target of negative peer experiences and the two patterns of HPA axis dysregulation, individual patterns of cortisol over the course of the day and HPA axis reactivity to stress. General life distress has been linked to a flattening of the diurnal pattern of cortisol during adolescence (Doane et al., 2013) and more specific to the current study, a composite measure of adolescent- and parent-reports of peer victimization was found to be associated with a similar flattened diurnal pattern (Knack et al., 2011). While one twin-study did find a steeper diurnal pattern for adolescents with higher rates of peer victimization (Brendgen et al., 2017), this study utilized difference scores between sibling pairs for all measures making it difficult to directly compare the findings with other studies. In addition, a dulled cortisol reaction to stress has also been found in response to experimentally manipulated socially stressful experiences. Cortisol reactivity in response to an experimental social stress test was blunted for those high in peer victimization in comparison to those low in peer victimization in two separate studies (Knack et al., 2011; Calhoun et al., 2014). It should be mentioned that a similar study did not find any association between cortisol reactivity in response to a social stress test and peer victimization (Hamilton et al., 2008) but unlike the other studies cited here, this study utilized retrospective reports of bullying from undergraduates reporting about being targeted for bullying as far back as elementary school making it difficult to compare findings across the studies.

Cortisol/HPA axis dysregulation and depression

Similarly, studies have found associations between our indirect effect and outcome measures; specifically, flat diurnal patterns and patterns of a blunted cortisol response to stress have been shown to be linked to depression and depressive symptoms suggesting that it is important to examine cortisol over the

course of the day, as we do in the current study. For instance, adolescents with a past major depressive disorder (MDD) episode have been found to have a flatter diurnal slope in comparison to adolescents with no history of MDD (Doane et al., 2013). In terms of cortisol reactivity, a number of studies with adolescents and young adults all using the experimental methods of inducing stress found a blunted cortisol response to stress for those with a life-time history of MDD compared to adolescents with no history of MDD (Hankin et al., 2010; Morris et al., 2014) with a meta-analysis suggesting that this was especially the case when the experiment took place in the afternoon (Burke et al., 2005). Other studies have found similar findings in maltreated children (Cicchetti et al., 2010; Harkness et al., 2011) and in typical adolescents (Rudolph et al., 2018). Finally, this pattern was observed with a more naturalistic method for measuring cortisol reactivity in a sample of adults. Using an experience sampling method to measure a stress reactivity, a similar method utilized in the current study, participants provided cortisol samples and reported about their experiences multiple times a day over the course of multiple days and it was found that there was a blunted cortisol response to the report of a negative event for clinically depressed participants compared to healthy controls (Peeters et al., 2003).

Other evidence of the indirect pathway

Two other studies have examined peer victimization, depressive symptoms, and cortisol simultaneously (Rudolph, Troop-Gordon, & Granger, 2011; Vaillancourt et al., 2011) and found similar associations between both peer victimization and cortisol and cortisol and depressive symptoms but neither study had goals for examining indirect effects and thus did not test this hypothesis. These findings along with the findings that show a consistent link between peer victimization and depression presented earlier in this review suggest that there may be a connection that runs between all three constructs, providing specific evidence of an indirect pathway. The overall pattern across these studies provided evidence of the indirect effect of HPA axis functioning (i.e. flattened

diurnal pattern of cortisol and dampened cortisol reactivity to a stressful experience) in the association between the association between peer victimization and depressive symptoms. In other words, these studies found that peer victimization was linked to HPA axis dysregulation and that this dysregulation was linked to depressive symptoms, as would be expected in an indirect pathway.

Current study

The goal of the current study is to provide preliminary evidence that HPA axis dysregulation as a possible mechanism for the reciprocal as- sociation between adolescent peer victimization and depressed affect. Towards completing this goal, participants completed self-report measures of peer victimization and symptoms of depressed affect once. Also, multiple times a day over multiple days, participants provided self- reports of negative experiences and saliva samples, both collected in conjunction to model HPA axis regulation in terms of cortisol diurnal daily patterns and cortisol reactivity. Compared with collecting one saliva sample per day or two measures over the course of one or two days, the current data collection method of cortisol allows for a more reliable measures of the daily cortisol pattern and a more naturalistic measure of cortisol reactivity in response to a negative event (in comparison to studies that use an experimental manipulation method). Two hypotheses were tested using the data from this study. In general, it is hypothesized that HPA axis functioning will have a significant indirect effect on the association between peer victimization and depressed affect. Given that a blunted/hyporeactive cortisol pattern has been shown to be associated with both peer victimization and depression, we specifically hypothesized that a hyporeactive cortisol pattern would be found for the indirect effect. Also, it was hypothesized that the measure of reactive dysregulation, in comparison to the diurnal measure of dysregulation, would have stronger indirect effects in the pathway given that reactivity is a more direct measure of HPA axis functioning in terms of the HPA axis being a system that responds to stressful experiences. Successfully testing these hypotheses

would provide the initial evidence that HPA axis dysregulation as a possible mechanism for the reciprocal association between adolescent peer victimization and depressed affect.

Method

Participants

A total of 113 boys (*n* = 61) and girls (*n* = 52) participants from grade 5 (*M* age = 10.31 years) and grade 6 (*M* age = 11.33 years) were recruited from a public school in a community near the greater Montreal metropolitan area. The population of the schools came from middle- class, English and French speaking neighborhoods and was predominantly European-Canadian. While English was not the first language of all participants, all instruction at the school was in English and students were fluent in English. A total of 134 consent letters were sent to the parents with an 85.07% (*n* = 114) return rate of the letters and an 84.33% (*n* = 113) participation rate. The institutional review board at the third author's institution approved the research described in this study.

Procedure

After receiving parental consent and child assent for participation, the participants completed two tasks (completing a booklet about their experiences 20 min earlier and providing saliva) five times per day for four consecutive school days (Tuesday through Friday). The five times were (a) 30 min after waking (*M* = 6:45 A.M., *SD* = 25.80 min), (b) fifteen minutes after entering the classroom at the start of school (*M* = 8:15 A.M., *SD* = 24.60 min), (c) fifteen minutes after returning to the classroom after a recess time (*M* = 10:00 A.M., *SD* = 29.40 min), (d) fifteen minutes after entering the classroom after the lunch period (*M* = 12:25 P.M., *SD* = 21.00 min), and then at the end of the school day (*M* = 2:00 P.M., *SD* = 16.20 min). To control for the effects of food, drink, and exercise on cortisol, study staff were present to remind/ensure participants did not eat or drink as well as engage in physical activity prior to each assessment. The post-

lunch assessment occurred 45 min after the end of the lunch period and 15 min after the end of a post-lunch free play period.

During each of the 5 daily time points for each day the participants completed two tasks: a booklet of their experiences and provided saliva samples. The participants first completed a booklet where they reported about their experiences 20 min previous and also how they felt about themselves at the moment. While completing the booklets each time, with the assistance of study staff participants used a passive drool procedure to expectorate 5 ml of saliva into a plastic vial to provide measures of cortisol. For the time points that took place at the school (i.e. all except the first assessment of the day), students completed the booklets in a classroom and study staff were present to help and provide instructions to the participants for completing the booklets and providing the saliva sample. In this respect, the study staff was able to provide a specific reference concerning the previous 20 min (e.g. “20 min ago was just before you came back to class from recess”). The vials were then sealed, placed into plastic bags that were coded for the day and time of day, temporarily stored in a container with dry ice, and then placed in long term storage. For the first daily time point, participants were verbally instructed (as well as given written instructions) about completing the task 30 min after waking the following morning and given a booklet, vial to provide saliva, and a plastic zip lock bag to transport the booklet and vial to school. Each participant provided up to 20 samples of saliva and reported 20 experiences (i.e., 5 samples per day for 4 days). Overall, 6.73% of the saliva samples were missing or fell above or below measurable thresholds for cortisol whereas 15.58% of experiences were not completed.

During the week prior to the experience sampling, participants filled out self-report measures of peer victimization and psychosocial functioning. A questionnaire designed to be completed in a 1-hour session during class time was used. In a group administration fashion, the questionnaires were given to the participating students during their homeroom class time. Three participants were not present during the day of the data collection. The final sample was

comprised of 110 students with complete data.

Participants were given $2 to bring back the consent form regardless of consent being provided or not and $20 for completing the study measures.

Measures

Experience sampling measures

To provide study measures, students completed booklets and pro- vided saliva five times per day over the course of four consecutive days. When completing the booklets each time, the participants were first instructed to report about experiences that had occurred 20 min previously. During each instance the time of day was recorded and later recoded as hours since waking (e.g. 2.5 h = two and one-half hours after waking).

Negativity of experience. For each experience, participants were prompted "How was the interaction with your friends)/classmate(s)?" and then asked, "How did you feel about it?" Participants' answers could range from 1, Very Positive, to 7, Very Negative (M = 3.29, S.D. = 1.94).

Cortisol. Salivary cortisol was assayed using a kit from DSL (DSL, Webster, Texas) with procedures modified to increase the sensitivity of the cortisol assay. Briefly, 50 μL of saliva were incubated with 50 μL of ^{125}I-cortisol and 50 μL of primary antibody and placed in a waterbath at 37 °C for two hours. A 500 μL PBS wash was added to the tubes prior to centrifugation. The pellet, representing the bound cortisol was then counted in a gamma counter and cortisol concentrations were calculated from a standard curve. The limit of detection for cortisol was 0.01 mg/dl, the intra- and inter-assay variability was 4.0% and 4.6%, respectively (on a range of 0.01–10 μg/dl dose). Analyses conducted to identify outliers in the distributions of the cortisol scores found only 6 instances (out of over 2000) assessments in which scores were more than 2.5 standard deviations from the mean at a particular time or day. These outliers were drawn in so that all scores were within a reasonable perimeter around the mean. Raw cortisol values were log transformed for normality (then adding 2.26

to each score so as to make all of the values positive; *M* = 1.27, *SD* =0.39). (Table 1).

Self-report surveys

Peer victimization and depressed affect symptoms. To assess self- reported peer victimization, three items (α = 0.83) were used ("Others do mean things to me", "Others try to hurt me", "I am called names by others") that were adapted from Hamburger et al. (2011). For depressed affect, three items (α = 0.60) were also assessed ("I am often sad", "I often don't feel like eating", "I feel that nothing will ever work out for me") adapted from the Child Depression Inventory (Kovacs, 1981). These series of items are introduced with the following instructions: "Now, we'd like to know about you. Read each description and tell us how well that description fits you. Check the box on the scale that best describes you." The scale for each item is as follows: Never True (1), Rarely True (2), Sometimes True (3), Often True (4), and Always True (5).

Statistical analyses

A multi-level structural equation modeling (MSEM) analytic frame- work was used to account for the within-subject effects found between days and time over the course of each day in addition to testing between- subjects indirect effects (Muth´en and Muth´en, 2006). Missing data were accounted for in the analyses by using full information maximum likelihood (FIML) estimation methods. First, the diurnal pattern of cortisol modeled the change in pattern cortisol of the course of the day for each individual by entering the linear and curvilinear effects of time into the level one model, while also accounting for day of the week (see Fig. 1a). The second model, which built upon the first, modeled cortisol reactivity by adding the variable of the negativity of the experience as an additional within-individual correlate (again Fig. 1a). Since the daily pat- terns in cortisol were already accounted for in this model, a significant effect of negativity indicated that the negativity of the experience predicted change above and beyond the individual's daily pattern in cortisol. The between-

subject differences in the change in cortisol due to the experience while controlling for the weekly and daily patterns is labeled as reactivity (Fig. 1b). At the between-individual level, we created a latent factor of depression symptoms using the three depression symptom items as indicators.

Table 1

Means and standard deviations of the study variables.

	Range	M	SD	n
Within-Individual Variables				
Cortisol (logged)	0-2.22	1.27	.39	2108
Day	0-3	1.5	1.12	2038
Time (hours since waking)	0-15.40	3.56	2.73	2038
Negative Peer Experience	1-7	3.29	1.94	1918
Between-Individual Variables				
Peer Victimization	1-5	2.35	1.10	110
Depressed Affect	1-5	2.14	.80	110

Indirect pathways were then created by regressing self-reported peer victimization onto the latent factor of depression symptoms and peer victimization onto one of the cortisol measures, depending the model (see Fig. 1a and 1b). Lastly, we modelled the indirect effect of cortisol reactivity on the association between self-reported peer victimization and the latent factor of depressed affect. Given that previous studies of peer victimization and internalizing issues have found curvilinear effects (e.g., Leadbeater and Hoglund, 2009), preliminary analyses tested such effects. Indeed, preliminary analyses for cortisol reactivity revealed that the model fit was greatly improved by adding the curvilinear effects (i. e., time squared) of peer victimization to the model when testing for indirect effects. Thus, for the reactivity indirect models, paths from the linear and curvilinear effects of peer victimization to the latent construct of depressed affect were added to the between-subjects portion of the model. Then, paths from the linear and curvilinear effects of victimization were added to the three cortisol measures (i.e. linear effect for time of day, curvilinear effect of

time of day and reactivity) were added to the model. Finally, tests for indirect effects were then conducted to test for that statistical significance of the potential indirect effects (Hayes and Preacher, 2010). The similar paths were tested for the diurnal effects of cortisol but only linear effects of victimization were tested in these models.

Finally, we tested for any effects of gender and age on cortisol reactivity and depressed affect with no significant associations. In addition, we tested for age and gender differences in the association between peer victimization and either cortisol reactivity or depressed affect with no significant effects emerging either. Lastly, in the final model (detailed below) we tested whether any of the effects differed once age and gender were again included in the models to no effect. Given these null effects, age and gender were not included in the final models reported below.

Results

Initial findings: building the indirect model

First, the unconditional model of cortisol (i.e. model with no predictors) was tested and it was found that 76.97% (variance component =0.117, z = 21.53, $p < .05$) of the variability was at the within-subject level (i.e. individual differences over time) while 23.03% (variance component =0.035, z = 6.19, p < .05) of the variability was at the between-subject level (i.e.: differences between participants).

Next, we built the initial within-subjects portion of the model for cortisol using time of day effect, the curvilinear time of day effect, and day of the week effect. As has been found in previous studies mentioned in the introduction, there was a significant linear (b = -0.115, z = 11.23, p < .05) and curvilinear (b =0.009, z = 6.02, p < .05) effect of cortisol over the course of the day (i.e. the diurnal pattern). The highest rates of cortisol were seen just after waking with a gradual curvilinear decrease in cortisol levels over the course of the day ending with a slight increase at the end of the school day. In addition to the diurnal effects, there was a significant effect for the day of the week with higher levels of

cortisol found earlier in the week than later in the week ($b = -0.071$, $z = 6.43$, $p < .05$).

To measure reactivity of cortisol to negative events, negativity of the experience was added as a random predictor to the model containing each of the three time variables (i.e. linear and curvilinear time of day and day of week). As expected, negativity of the experience was positively associated with cortisol ($b = 0.059$, $z = 4.64$, $p < .05$). In other words, negative experiences predicted increases in cortisol above and beyond daily and weekly pattern indicating a reactivity response of cortisol in reaction to the negativity of the experience.

The between-subjects portion of the model testing the association between the predictor and the outcome was then added to depressed affect. As would be expected, more self-reported peer victimization was related to higher depressed affect ($b = 0.51$, $z = 5.09$, $p < .05$, $R^2 = 64.96\%$).

Pathways for cortisol diurnal effects

The between-subjects effect of peer victimization ($b = 0.018$, $z = 0.98$, $p > .05$) was not associated with the diurnal effects of cortisol. Additionally, diurnal effects of cortisol ($b = -1.197$, $z = 3.01$, $p > .05$) were not found to be associated with symptoms of depressed affect. Since the diurnal effects of cortisol were not associated with either the predictor or the outcome measures, indirect effects were not tested.

Pathways for cortisol reactivity

As seen in Fig. 2, between-subjects linear ($b = 0.038$, $z = 2.66$, $p < .05$) and curvilinear effects ($b = -0.007$, $z = -2.70$, $p < .05$) of peer victimization were found to be associated with cortisol reactivity.

Reactivity increased from low to average levels of peer victimization and sharply decreased as peer victimization increased from average to high levels of peer victimization with the lowest levels of reactivity at the highest levels of peer victimization (see Fig. 3). The linear and curvilinear effects of victimization

explained 7.08% of the variability in cortisol reactivity.

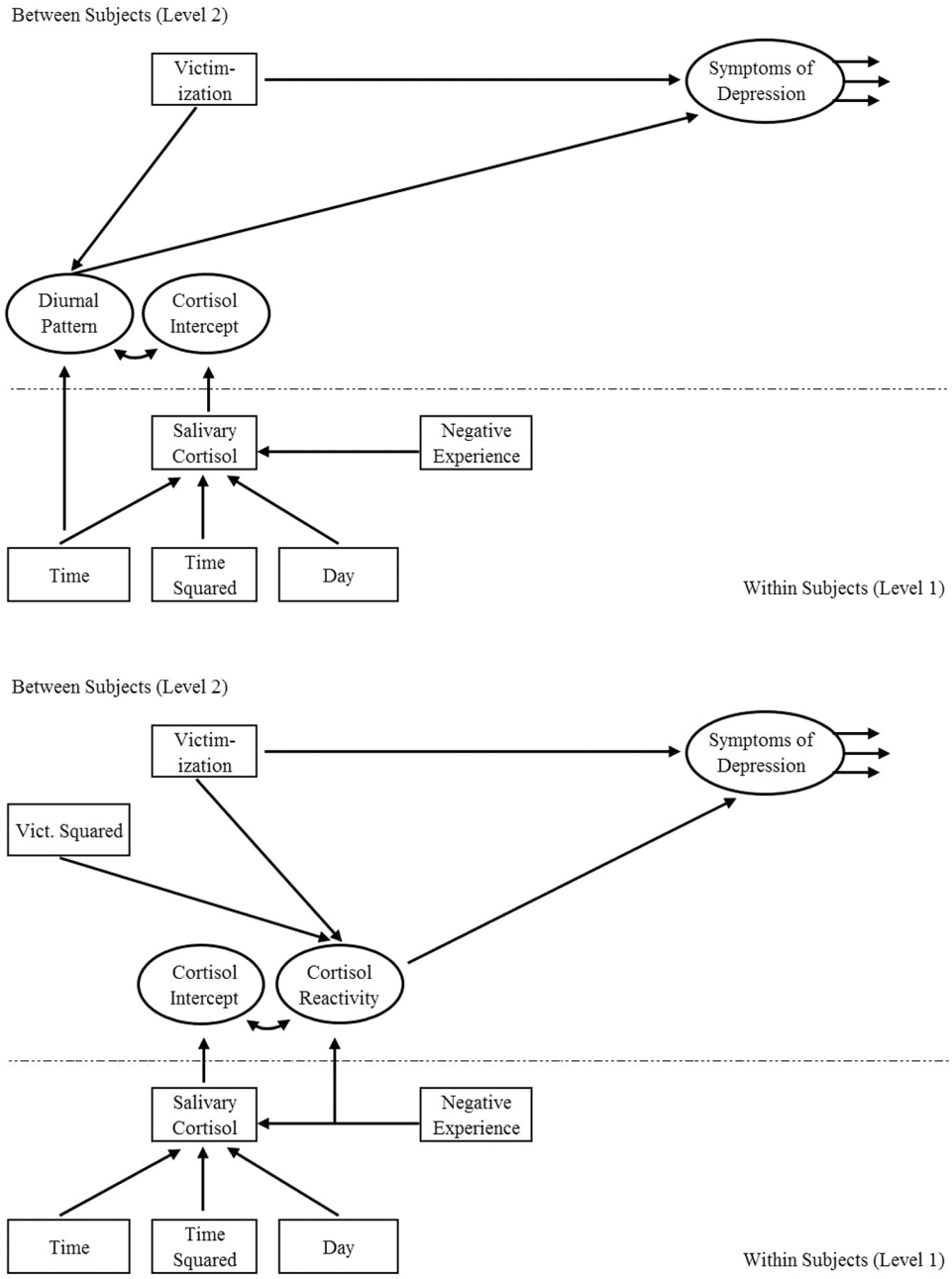

Fig. 1. a. Statistical model that tested the potential indirect effect of the diurnal pattern of cortisol on the association between peer victimization and symptoms of depression. b. Statistical model that tested the potential indirect effect of cortisol reactivity on the association between peer victimization and symptoms of depression.

Next, cortisol reactivity (b = -10.24, z = -2.03, p < .05) was found to be associated with depressed affect. To explain, lower cortisol reactivity was associated with higher rates of symptoms of depressed affect. Modeling the effects of the cortisol reactivity on depressed affect reduced variability in depressed affect by 52.99%.

Tests of indirect effects found linear and curvilinear effects of peer victimization on symptoms of depressed affect were partly through cortisol reactivity (b = -0.077, z = -2.29, p < .05). All told, the final model reduced variability in depressed affect by 86.67%.

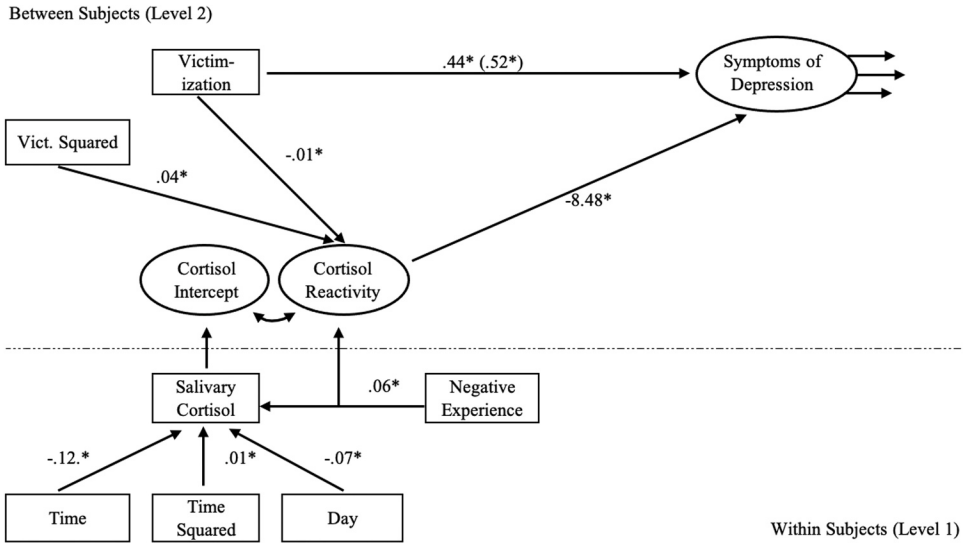

Fig. 2. Final model for cortisol reactivity as an indirect variable of the associations between peer victimization and depressive symptoms.

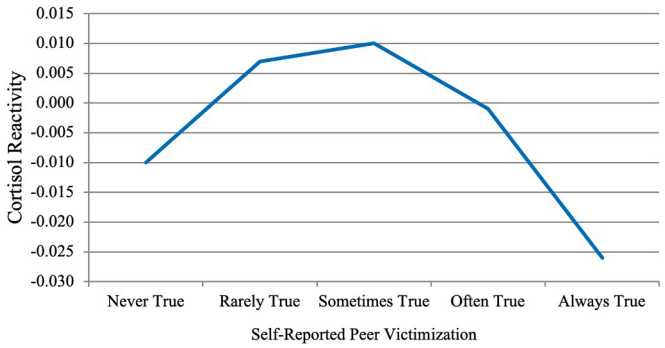

Fig. 3. Effect of self-reported peer victimization on cortisol reactivity in response to negative peer experiences.

Discussion

The goal of the current study is to provide preliminary evidence that HPA axis dysregulation as a possible mechanism for the reciprocal as- sociation between adolescent peer victimization and depressed affect using two measures of HPA axis dysregulation as indirect variable in the association between peer victimization and depressed affect. Only cortisol reactivity (i.e. increases in cortisol in response to a negative event), and not diurnal levels of cortisol, was found to have indirect effects for this association. Specifically, higher rates of peer victimization was associated with blunted cortisol reaction to negative events and this hypo-reactivity effect was associated with higher rates of depressed affect. Many separate studies of peer victimization and of depression have found similar associations with blunted cortisol but this is the first to test cortisol reactivity outside an experimental setting and the first to directly test the indirect effect of HPA axis dysregulation. Thus, this finding provides preliminary evidence of the HPA-axis dysregulation as a possible mechanism for future, more methodologically rigorous and resource intensive studies to understand the long-term impact of peer victimization and other negative peer experiences on depression.

In these findings, it was striking that it was only at higher rates, and not at average rates, of peer victimization where hypo-reactivity was associated with peer victimization as seen in a previous study (Vaillancourt et al., 2008). Given the stability of peer victimization for those reporting high rates of peer

victimization (Nylund et al., 2007), this finding may reflect the general development of HPA axis dysregulation where initial exposure to stressful, chronic events are linked to a hyper-reactive HPA axis and over time after continued exposure trans- forms to a hypo-reactive system (Cicchetti and Rogosch, 2001; Miller et al., 2007; Trickett et al., 2010). In this respect, this curvilinear effect suggests that hypo-reactive HPA axis may be only found at the extreme ends of the spectrum of those who experience high rates of negative peer experiences over time and given the indirect effects in the current study, this also suggests that when it comes to understanding outcomes of peer victimization, especially depressive symptoms, it may be import to put a special focus on those who are chronically experiencing peer victimization over time. This means that outcome studies may want to focus on groups that have characteristics that put them at risk for peer victimization and for depressive symptoms, such as those who are obese and high functioning individuals with autism spectrum disorders.

In terms of understanding depressive symptoms, the indirect effects point to the importance of examining the development of a hypo- reactive HPA axis. It has been previously suggested that a hypo- reactive HPA axis indicated that the system has been “scarred” and that this decreased reactivity of the system may put individuals at-risk for recurring depressive episodes over the course of their lifetime (Wichers et al., 2010) but how this system specifically develops is not known. Although the findings from the current study showed that stressful experiences such as peer victimization, a hypo-reactive HPA axis, and depressed affect make up a clear pathway, the order and timing of each component in the system and the development of the dysregulated HPA axis is not determined in the current study. It could be that chronic experiences of peer victimization over time could lead to a dysregulated HPA-axis which then leads to depressed affect but it could just as well be that peer victimization directly leads to depression and the dysregulated system is a result of the depression. Additionally, it may be that this specific form of dysregulation is not a pathway to depression but actually part of depression, a physiological process of

depression. To determine the specifics of the development of this pathway, longitudinal studies that measures chronic stressful experiences to capture the stability of such experiences as well cortisol reactivity to such experiences over the course of the day for multiple days must be conducted. Given that few individuals, in general, experience chronic negative experiences over time and the large resources required to conduct such a study, it may be more feasible to focus initial efforts on the high-risk groups for peer victimization and depression to increase the probability of capturing the development of such dysregulation occurring.

The current findings also suggest that interventions for negative peer experiences should obviously address reducing such experiences and also, address the outcomes of these negative experiences. Based on the current findings, again, it seems that such interventions may want to focus on those who are chronically targeted for such experience given that a dysregulated HPA axis most likely does not occur after one or two isolated negative peer experiences. These findings also suggest that we may want to identify ways to mitigate the initial hyper-reactivity of cortisol found when individuals initially are exposed to chronically stressful events, before the HPA-axis become hypo-reactive (Cicchetti and Rogosch, 2001; Miller et al., 2007; Trickett et al., 2010). A previous study found that the presence of a best friends during a negative experience buffered the spike in cortisol reactivity after a negative event suggesting that certain qualities of close friendships could help to pro- vide such protection to this axis (Adams et al., 2011). Much more research is needed to find out what specific aspects of friendships may specifically result in such protective effects in terms of cortisol reactivity but how a friend of an individual who is targeted for peer victimization actually responses to the victimization may provide insights. Specifically, a study on friends' reactions to being targeted for peer victimization (Adams et al., 2016) found that for those adolescents that thought their best friend would responded with statements that served to protect the self-worth and well-being of the adolescent if the adolescent was targeted for peer victimization that there was no association between peer victimization

and depressive symptoms (compared to adolescents whose friends were unlikely to respond in such a way). Additionally, the association between peer victimization and depressive symptoms was strengthened for those who thought their friends would respond in a negative manner (e.g. laugh at them) if they found out that the adolescent was victimized.

Additionally, an effect was found for day of the week where rates of cortisol was higher at the beginning of the week than at the end of the week. We could find no other study with similar findings. This finding may be an artifact of the assessment procedure. The initial experience of providing a saliva sample may have led to a mild arousal of the HPA axis that likely decreased across the week as the task became more familiar. Alternatively, this finding could indicate that students have higher rates of stress at the beginning of the school week than at the end of the school week. Future studies that take place over the course of multiple school weeks are needed to determine which hypothesis is correct.

A few issues need to be considered when interpreting the current findings. First, as we have noted already, the current study cannot determine the direction of the indirect pathway. Given that the associations between peer victimization and depressive symptoms have been shown to be bi-directional (Reijntjes et al., 2010), it could be that depressive affect might lead to peer victimization. In this respect, the current study is only testing indirect effects in a statistical sense rather than in a methodological manner (i.e., where the predictor leads to the mediator which in turn, leads to the outcome). Also, the current study used abbreviated measures of both peer victimization and depressive symptoms. While we did find effects using these shortened measures, future studies will want to use more expanded measure to be able to distinguish the effects of different types of peer victimization, to ensure sensitivity to changes in depressive symptoms in future longitudinal studies, to test similar effects on other forms of internalizing problems such as anxiety, and to be able to compare findings with previous studies that use the full-scale measures. Additionally, our measure of the diurnal pattern of cortisol was abbreviated in that we had no measures of cortisol after the end of the school

day and included no measures of evening cortisol. While we were able to capture the curvilinear effect of the diurnal pattern with the current data, the abbreviated diurnal measurement of cortisol could be a possible explanation for the null effects found for this measure. Thus, one should be careful, as is the case of all null findings, interpreting the lack of findings here and suggest that future studies will incorporate measures of evening cortisol. Similar points should be mentioned about the cortisol awaking response, which the current study did not measure due to our use a school-based, naturalistic methods and our focus on measuring cortisol reactivity. Given the important role of this awaking response in understanding the HPA axis regulation, future studies should work to incorporate the appropriate protocols to capture this response.

Declarations of Interest

none.

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References

Adams, R.E., Fredstrom, B.K., Peets, K.K., Hodges, E.V.E., Bowker, J.C., Holleb, L.J., Gilman, R., 2016. Validating a measure of friends' responses to self-disclosure in adolescent obese and public school samples. J. Clin. Child Adolesc. Psychol. https:// doi.org/10.1080/15374416.2015.1094738.

Adams, R.E., Santo, J.B., Bukowski, W.M., 2011. The presence of a best friend buffers the effects of negative experiences. Dev. Psychol. 47, 1786–1791.

Brendgen, M., Ouellet-Morin, I., Lupien, S., Vitaro, F., Dionne, G., Boivin, M., 2017. Environmental influence of problematic social

relationships on adolescents' daily cortisol secretion: a monozygotic twin-difference study. Psychol. Med. 47 (3), 460–470. https://doi.org/10.1017/S003329171600252X.

Burke, H.M., Davis, M.C., Otte, C., Mohr, D.C., 2005. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 30 (9), 846–856. https://doi.org/10.1016/j.psyneuen.2005.02.010.

Calhoun, C.D., Helms, S.W., Heilbron, N., Rudolph, K.D., Hastings, P.D., Prinstein, M.J., 2014. Relational victimization, friendship, and adolescents' hypothalamic-pituitary-adrenal axis responses to an in vivo social stressor. Dev. Psychopathol. 26 (3), 605–618. https://doi.org/10.1017/S0954579414000261.

Cicchetti, D., Rogosch, F.A., 2001. Diverse patterns of neuroendocrine activity in maltreated children. Dev. Psychopathol. 13 (3), 677–693.

Cicchetti, D., Rogosch, F.A., Gunnar, M.R., Toth, S.L., 2010. The differential impacts of early physical and sexual abuse and internalizing problems on daytime cortisol rhythm in school-aged children. Child Dev. 81 (1), 252–269. https://doi.org/ 10.1111/j.1467–8624.2009.01393.x.

Copeland, W.E., Wolke, D., Angold, A., Costello, E.J., 2013. Adult psychiatric outcomes of bullying and being bullied by peers in childhood and adolescence. JAMA Psychiatry 70 (4), 419–426.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130 (3), 355–391.

Doane, L., Mineka, S., Zinbarg, R., Craske, M., Griffith, J., Adam, E., 2013. Are flatter diurnal cortisol rhythms associated with major depression and anxiety disorders in late adolescence? The role of life stress and daily negative emotion. Dev. Psychopathol. 25 (3), 629–642. https://doi.org/10.1017/S0954579413000060.

Guerry, J.D., Hastings, P.D., 2011. In search of HPA axis dysregulation

in child and adolescent depression. Clin. Child Fam. Psychol. Rev. 14, 135–160. https://doi.org/ 10.1007/s10567-011-0084-5.

Hamburger, M.E., Basile, K.C., Vivolo, A.M., 2011. Measuring Bullying Victimization, Perpetration, and Bystander Experiences: A Compendium of Assessment Tools. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, Atlanta, GA, p. 2011.

Hamilton, L.D., Newman, M.L., Delville, C.L., Delville, Y., 2008. Physiological stress response of young adults exposed to bullying during adolescence. Physiol. Behav. 95 (5), 617–624.

Hankin, B.L., Badanes, L.S., Abela, J.R.Z., Watamura, S.E., 2010. Hypothalamic pituitary adrenal axis dysregulation in dysphoric children and adolescents: cortisol reactivity to psychosocial stress from preschool through middle adolescence. Biol. Psychiatry 68 (5), 484–490. https://doi.org/10.1016/j.biopsych.2010.04.004.

Harkness, K.L., Stewart, J.G., Wynne-Edwards, K.E., 2011. Cortisol reactivity to social stress in adolescents: role of depression severity and child maltreatment. Psychoneuroendocrinology 36 (2), 173–181.

Hawker, D.S.J., Boulton, M.J., 2000. Twenty years' research on peer victimization and psychosocial maladjustment: a meta-analytic review of cross-sectional studies. J. Child Psychol. Psychiatry 41 (4), 441–455.

Hayes, A.F., Preacher J., K., 2010. Quantifying and testing indirect effects in simple mediation models when the constituent paths are nonlinear. Multivar. Behav. Res. 45, 627–660. https://doi.org/10.1080/00273171.2010.498290.

Knack, J.M., Jensen-Campbell, L.A., Baum, A., 2011. Worse than sticks and stones? Bullying is associated with altered HPA axis functioning and poorer health. Brain Cogn. 77 (2), 183–190.

Kovacs, M., 1981. Rating scales to assess depression in school- aged children. Acta Paedopsychiatr. 46, 305–315.

Leadbeater, B.J., Hoglund, W.L., 2009. The effects of peer victimization and physical aggression on changes in internalizing from first to third grade. Child Dev. 80 (3), 843–859.

Menke, A., 2019. Is the HPA axis as target for depression outdated, or is there a new hope? Front. Psychiatry 10, 101.

Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. 133 (1), 25–45.

Morris, M.C., Rao, U., Wang, L., Garber, J., 2014. Cortisol reactivity to experimentally manipulated psychosocial stress in young adults at varied risk for depression. Depress Anxiety 31 (1), 44–52.

Muth´en, L.K., Muth´en, B.O., 2006. Mplus: Statistical Analysis with Latent Variables, 4th ed. Muth´en & Muth´en, Los Angeles, CA.

Nylund, K., Bellmore, A., Nishina, A., Graham, S., 2007. Subtypes, severity, and structural stability of peer victimization: what does latent class analysis say? Child Dev. 78 (6), 1706–1722.

O'Keane, V., Frodl, T., Dinan, T.G., 2012. A review of atypical depression in relation to the course of depression and changes in HPA axis organization. Psychoneuroendocrinology 37 (10), 1589–1599.

Peeters, F., Nicholson, N.A., Berkhof, J., 2003. Cortisol responses to daily events in major depressive disorder. Psychosom. Med. 65 (5), 836–841.

Rudolph, K.D., Troop-Gordon, W., Modi, H.H., Granger, D.A., 2018. An exploratory analysis of the joint contribution of HPA axis activation and motivation to early adolescent depressive symptoms. Dev. Psychobiol. 60 (3), 303–316.

Reijntjes, A., Kamphuis, J.H., Prinzie, P., Telch, M.J., 2010. Peer victimization and internalizing problems in children: a meta-analysis of longitudinal studies. Child Abus. Negl. 34 (4), 244–252. https://doi.org/10.1016/j.chiabu.2009.07.009.

Takizawa, R., Maughan, B., Arseneault, L., 2014. Adult health outcomes of

childhood bullying victimization: evidence from a five-decade longitudinal British birth cohort. Am. J. Psychiatry 171 (7), 777–784.

Trickett, P.K., Noll, J.G., Susman, E.J., Shenk, C.E., Putnam, F.W., 2010. Attenuation of cortisol across development for victims of sexual abuse. Dev. Psychopathol. 22 (1), 165–175.

Vaillancourt, T., Duku, E., Decatanzaro, D., Macmillan, H., Muir, C., Schmidt, L.A., 2008. Variation in hypothalamic-pituitary-adrenal axis activity among bullied and non- bullied children. Aggress. Behav. 34, 294–305.

Vaillancourt, T., Duku, E., Becker, S., Schmidt, L.A., Nicol, J., Muir, C., MacMillan, H., 2011. Peer victimization, depressive symptoms, and high salivary cortisol predict poorer memory in children. Brain Cogn. 77 (2), 191–199.

Vaillancourt, T., Hymel, S., McDougall, P., 2013. The biological underpinnings of peer victimization: understanding why and how the effects of bullying can last a lifetime. Theory Pract. 52 (4), 241–248.

Wichers, M., Geschwind, N., Van Os, J., Peeters, F., 2010. Scars in depression: is a conceptual shift necessary to solve the puzzle? Psychol. Med. 40 (3), 359–365.