A Discordant Monozygotic Twin Design Shows Blunted Cortisol Reactivity Among Bullied Children

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Objective: Childhood adverse experiences are known to engender persistent changes in stress-related systems and brain structures involved in mood, cognition, and behavior in animal models. Uncertainty remains about the causal effect of early stressful experiences on physiological response to stress in human beings, as the impact of these experiences has rarely been investigated while controlling for both genetic and shared environmental influences. **Method:** We tested whether bullying victimization, a repeated adverse experience in childhood, influences cortisol responses to a psychosocial stress test (PST) using a discordant monozygotic (MZ) twin design. Thirty pairs (43.3% males) of 12-year-old MZ twins discordant for bullying victimization were identified in the Environmental Risk (E-Risk) Longitudinal Twin Study, a nationally representative 1994–1995 cohort of families with twins. **Results:** Bullied and nonbullied MZ twins showed distinct patterns of cortisol secretion after the PST. Specifically, bullied twins exhibited a blunted cortisol response compared with their nonbullied MZ co-twins, who showed the expected increase. This difference in cortisol response to stress could not be attributed to children's genetic makeup, their familial environments, pre-existing and concomitant individual factors, or the perception of stress and emotional response to the PST. **Conclusion:** Results from this natural experiment provide support for a causal effect of adverse childhood experiences on the neuroendocrine response to stress. J. Am. Acad. Child Adolesc. Psychiatry, 2011;50(6):574–582. **Key words:** early-life stress, cortisol, HPA axis, discordant MZ twin design, bullying

Severe abuse and neglect experienced early in life are associated with poor physical and mental health.[1] Harmful effects of other forms of stress experienced in childhood, such as bullying victimization, are also increasingly recognized, but their consequences for health are less studied. Bullying is present when children or adolescents are exposed to repeated harassment and humiliation from peers between whom there is an imbalance of power whereby it is difficult for the victims to defend themselves. Evidence indicates that emotional problems in bullied children were not merely due to the victims' genetic background or pre-existing characteristics,[2] supporting an environmentally mediated effect of bullying victimization on emotional problems. Considering that approximately 13% of children are victims of bullying worldwide,[3] and that its adverse impacts are not entirely explained by genetic factors, identifying the mechanisms by which bullying victimization gets "under the skin" is pressing.[4] This is the focus of the present study.

It has been hypothesized that early-life stress may alter physical and mental health through hypothalamic–pituitary–adrenocortical (HPA) axis activity.[5,6] The HPA axis underlies both adaptive and maladaptive responses to stress. Adaptive responses are characterized by a relatively rapid increase in cortisol, the end-product of the HPA axis, followed by a progressive decline. Conversely, persistently increased or

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blunted cortisol secretion may signal maladaptive responses to stress and are hypothesized to increase vulnerability to stress-related diseases.[7] Whether bullying victimization, a repeated stress commonly experienced during childhood, is associated with disrupted cortisol secretion remains unclear.[8-10]

Early-life stress, such as maternal depression and maltreatment, has been associated with disrupted HPA axis activity, showing both high *and* low cortisol secretion in childhood.[5,11] A study reported that 12- to 16-year-old females with a history of maltreatment showed blunted cortisol responses to the Trier Social Stress Test (TSST) in comparison to typical increases exhibited by controls matched for age and neighborhood.[12] Studies examining the association between early-life stress and cortisol activity, however, often rely on retrospective reports of childhood adversity, which may include bias and substantial measurement error. In addition, because most studies are cross-sectional, it is difficult to test the cumulative cost of repeated adverse experiences. Finally, time delay between early-life stress and cortisol measurements leaves the possibility that intermediate events obscure the presumed effects of early-life stress on HPA axis activity.

Animal models have demonstrated that early-life stress causes persistent changes in HPA axis activity that can not be explained by genetic factors.[11,13,14] In human studies, the effect of early-life stress on the HPA axis, controlling for both genetic and shared environmental influences, has rarely been investigated.[15] Disrupted cortisol secretion in bullied children could be explained by inherited factors affecting both cortisol activity and exposure to adversity because cortisol reactivity and bullying victimization are partly heritable.[16-19] In the absence of experimental designs involving random assignment of participants to different early stress conditions, uncertainty remains about the causal effect of early-life stress on cortisol secretion in human beings.

Randomly assigning children to adverse environments is unethical in human beings for obvious reasons, hence limiting causal inferences relating to early-life stress. Alternatively, rigorous control for confounders could be achieved by contrasting genetically identical individuals drawn from the same family environment but who are exposed to distinct naturally occurring experiences. The discordant monozygotic (MZ) twin design offers this possibility. Differences in cortisol activity within MZ (genetically identical) twins who grow up in the same family but who are exposed to different experiences such as bullying victimization would not be attributable to the children's genetic makeup or their familial environments.[15,20]

The objective of this study was to examine the impact of bullying victimization on cortisol reactivity. More specifically, we examined whether cortisol response to a psychosocial stress test (PST) differed between bullied and nonbullied children controlling for the confounding effect of genetic and familial environmental factors using a discordant MZ twin design. Based on previous findings suggesting that early-life stress is associated with abnormal cortisol secretion, we hypothesized that bullied twins would have impaired cortisol responses to the PST whereas their nonbullied MZ co-twins will show an increase in cortisol secretion after exposure to this experimental stress task. We also explored the association between cortisol response and a continuously distributed index of bullying in bullied twins to investigate whether children exposed to more frequent, chronic, and severe bullying experiences showed greater cortisol disruption during the PST.

METHOD

Sample

Participants were recruited from the E-Risk Longitudinal Twin Study, which tracks the development of a nationally representative birth cohort of 2,232 British children.[21] The sample was drawn from a larger birth register of twins born in England and Wales in 1994–1995. The E-Risk sample was constructed in 1999–2000, when 1,116 families with same-gender 5-year-old twins (93% of those eligible) participated in home-visit assessments. Follow-up home visits were conducted when the children were aged 7 (98% participation), 10 years (96% participation), and 12 years (96% participation). Twins' zygosity was determined with a standardized zygosity questionnaire that has been shown to have 95% accuracy.[22] Ambiguous cases were zygosity typed using DNA. Ethical approval was granted by the Joint South London and Maudsley and the Institute of Psychiatry NHS Ethics Committee (UK). Parents gave informed consent and children gave assent to participate in the study.

Based on prior investigations conducted by our research group, 27% of the variance in bullying victimization was due to unique environments or random experiences.[19] These factors may explain why geneti-