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Heritability of daytime cortisol levels and cortisol reactivity in children

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Summary Individuals differ widely in cortisol output over the day and cortisol reactivity to challenge, both of which are relevant to disease risk. There is limited evidence concerning the heritability of these differences, so we evaluated the heritability of cortisol levels in the afternoon and cortisol reactivity using a twin design. The study involved 80 monozygotic (MZ) and 70 dizygotic (DZ) same-sex twin pairs aged 11.2 years on average. Salivary cortisol was measured in the afternoon at home before and after playing a computer game. Ratings of excitement and upset were also obtained, and objective task performance was assessed. Salivary cortisol levels averaged 4.08 (S.D. 2.3) nmol/l at pretask baseline, and declined on average over the session to 3.45 (1.9) nmol/l immediately after the tasks and 2.87 (1.6) nmol/l 10 min later. There were, however, marked individual differences, with cortisol reactivity (difference between pretask baseline and post-task 1) ranging from +4.53 to −6.23 nmol/l. Intra-class correlations for all the cortisol parameters were substantially greater for MZ (range 0.41—0.57) than for DZ (0.11—0.29) twin pairs. Quantitative genetic modelling confirmed significant heritability for pretask baseline cortisol (58%), the two post-task values (60 and 56%), and cortisol reactivity (44%). The study lacked power for assessing sex differences. Subjective reports of excitement were also somewhat heritable, but there was little covariation of cortisol and subjective responses, so genetic influences on covariation could not be tested. These findings indicate that individual differences in children's cortisol levels recorded before tasks and cortisol reactivity to behavioural challenges are influenced by genetic factors.
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1. Introduction

There are marked individual differences both in cortisol levels over the day and cortisol responses to behavioural challenge. These differences are thought to be relevant to a

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range of pathologies, including depression, abdominal adiposity, cognitive impairment in old age, hypertension, autoimmune disease and resistance to infection (Bjorntorp, 2001; Herbert et al., 2006; McEwen, 2007). Individual differences are determined by a range of factors including the perinatal environment (Meaney, 2001; Phillips, 2007), early childhood adversity (Heim et al., 2000), and psychosocial factors such as stress exposure, social support and psychological traits (Miller et al., 2007). Polymorphisms of genes regulating glucocorticoid and mineralocorticoid receptor function are associated with cortisol responsivity (Wüst et al., 2004; DeRijk et al., 2006). Nevertheless, the contribution of genetic factors to cortisol variation in the population may vary with factors such as timing of assessments and whether cortisol is measured under resting conditions or in response to challenge. Bartels et al. (2003a) reported a meta-analysis of five twin studies in which the heritability of 'basal' cortisol was put at 62%, but their analysis conflated measures taken under resting conditions in the morning and the cortisol awakening response (CAR), the increase in cortisol that typically occurs over the first 30—45 min after waking. Wüst et al. (2000) observed significant heritability of the CAR but not cortisol over the remainder of the day in a study of 52 monozygotic (MZ) and 52 dizygotic (DZ) twin pairs. This pattern was replicated in a larger study of 199 MZ and 272 DZ adult twin pairs, in which the CAR showed round 30% heritability, with no significant effects for values recorded later in the day (Kupper et al., 2005). A mixed pattern was recorded in a study of 180 pairs of 12-year-old twins, with significant heritability for samples taken early in the day and at noon, but not in the evening (Bartels et al., 2003b). A report from the Wisconsin twin project showed no heritability for samples taken in the afternoon in younger children (average age 8.64 years) (Schreiber et al., 2006). These findings suggest that cortisol levels soon after waking and early in the day are heritable, while resting cortisol levels later in the day are not. Studies of the heritability of cortisol reactivity to behavioural challenge have been inconsistent (Kirschbaum et al., 1992). However, Federenko et al. (2004) demonstrated that heritability of cortisol reactions to a standard stress battery increased with repeated exposure, so may depend on the context of task presentation. A study of 19-month-old twins has suggested that cortisol reactivity to unfamiliar situations was more heritable in infants who had not experienced familial adversity than in those with risk factors such as low birth weight, low socioeconomic status (SES), and maternal hostile behaviours (Ouellet-Morin et al., 2008).

The majority of studies of cortisol over the day have relied on participants being provided with sampling devices and collecting saliva samples at predetermined times without supervision. This may result in additional error, since respondents will be in diverse situations, and not all samples are reliably taken at the required times (Kudielka et al., 2003). Since cortisol levels vary over the day, and the twins may not collect their samples at the same times, heritability could be underestimated. In the present study, we tested a sample of young twins in their own homes under standardised conditions, with simultaneous cortisol measures from each member of the pair obtained by the research team. In addition to measuring baseline levels, we sampled cortisol after the administration of a computer game. The game was not intended to provoke stress, but to act as a behavioural challenge that would elicit individual differences in cortisol responsivity. Computer games have been widely used in young children to stimulate physiological responses, and the cardiovascular responses to computer games have been shown to relate to future risk of high blood pressure (Treiber et al., 2001). The challenging nature of the game was assessed by taking ratings of excitement, and we checked whether or not distress was elicited by obtaining ratings of upset. These subjective measures also provided an opportunity to assess covariation in endocrine and subjective responses. Recent molecular genetic studies have suggested that polymorphisms in enzymes regulating monoamine neurotransmitter pathways may affect both endocrine and subjective responses to psychological stress (Jabbi et al., 2007). We reasoned that if cortisol is heritable, this could be due either to genetic influences on primary mechanisms within the hypothalamic-pituitary-adrenocortical (HPA) axis, or to shared genetic influences on emotional and cortisol responses. We therefore assessed the heritability of subjective responses, and evaluated the covariation of cortisol and subjective responses. If there was significant covariation, we planned to carry out bivariate modelling to determine the extent to which genetic and environmental factors accounted this covariation.

2. Method

2.1. Participants

Participants in this study were part of the Twins' Early Development Study (TEDS), a population-based cohort of twins born in the UK in 1994, 1995, and 1996. The TEDS cohort is reasonably representative of population demographics, as described elsewhere (Oliver and Plomin, 2007). Zygosity was assessed through a parent questionnaire of physical similarity, which has been shown to be over 95% accurate when compared with DNA testing (Price et al., 2000). Where zygosity was unclear from the questionnaire, DNA testing was conducted. The subsample tested in this investigation was part of the follow-up in a substudy primarily concerned with genetic and environmental determinants of childhood adiposity and eating behaviours (Wardle et al., 2008). The sample consisted of same-sex twins, so that the DZ could be matched with MZ twin pairs. 173 families with same-sex twins were visited at home for this study and cortisol data were collected from 150 pairs, consisting of 30 MZ male (MZM), 50 MZ female (MZF), 30 DZ male (DZM) and 40 DZ female (DZF) pairs. There were no differences in the characteristics of participants who did and did not provide cortisol samples. This study was approved by the research ethics committees of Kings College London and University College London, and every child's parents provided written informed consent.

2.2. Procedure

Families were visited at home by trained researchers. Most sessions (71%) were held after 1530 h, but 29% took place at mid-day, starting between 1200 and 1330 h. None of the children had eaten within 3 h of the cortisol assessment. Children's heights were measured to the nearest 1.0 mm with

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