Daytime Cortisol Secretion in 6-Month-Old Twins: Genetic and Environmental Contributions as a Function of Early Familial Adversity

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Background: Dysregulation of daytime cortisol activity has been associated with stress-related pathologies. Research suggests that early environmental adversity might shape cortisol activity. However, little is known about the genetic and environmental contributions to early cortisol and how this varies as a function of environmental circumstances. The goals of the study were to estimate the genetic and environmental contributions to daytime cortisol secretion in infant twins and to investigate whether these contributions varied as a function of familial adversity (FA).

Methods: Participants were 517 6-month-old twins. Salivary cortisol was collected when the infants woke up at home and in the morning, upon arrival at the laboratory. Familial adversity was defined by seven perinatal and postnatal risk factors: maternal smoking during pregnancy, low birth weight, low family income, low maternal education, single parenthood, young motherhood, and maternal hostile/reactive behaviors. Genetic and environment contributions to cortisol activity were estimated for high (three risk factors or more: 21.3% of the sample) versus low FA.

Results: Genetic factors accounted for cortisol levels in different ways: a moderate "main effect" of genes was found for home-based awakening cortisol, whereas the contribution of genes to morning cortisol was conditional to FA. Genetic factors accounted for most of the variance in morning cortisol in high family adversity but not in low family adversity.

Conclusions: Early FA modulates the heritability of morning cortisol in infants. The results are consistent with the diathesis-stress model, with genetic factors more likely to be expressed in adverse settings.

Key Words: Cortisol, early adversity, genetic–environment interaction (G×E), HPA axis, stress, twin study

The Hypothalamus-Pituitary-Adrenal (HPA) axis activity underlies the organism's response to stressful conditions (1). Cortisol, the end-product of the HPA axis, peaks shortly after awakening and progressively decreases throughout the day. This circadian cycle is established within the first months of life (2–4). Whereas cortisol generally helps the organism face daily life obligations, disturbed patterns of cortisol secretion are potentially detrimental in the long run (1). Dysregulation of daytime cortisol activity has indeed been associated with stress-related pathologies, including depression (5,6), post-traumatic stress disorder (7,8), anxiety (9), externalizing behaviors (10), obesity (11), and cognitive deficits (12,13). Describing the causes of early cortisol secretion is thus an important step in understanding the vulnerability to later stress-related diseases.

Research has documented an association between disrupted daytime cortisol and markers of adversity, such as low familial socioeconomic status (14), economic poverty (15), single motherhood (16), low birth weight (17), prenatal alcohol and cigarette exposure (18), neglect (19,20), and abuses (21–24). These findings suggest that early adversity might shape cortisol activity (25–30). However, because these studies did not consider the role of genetic factors in predicting cortisol, the role of environmental adversity is still open to debate (31–33).

Individual differences in daytime cortisol levels likely arise from the joint contribution of genetic and environmental factors. A handful of twin studies have examined the genetic–environmental etiology of daytime cortisol activity. They suggest a substantial heritability and no shared environment contribution to daytime cortisol (34) (35–39). However, most of these studies relied on older and age-heterogeneous samples of twins. Due to the plasticity of the yet immature but fast-developing brain structures during the first years of life, early environmental adversity could influence cortisol activity in decisive ways (40,41). Accordingly, the present study focused on the gene environment processes underlying daytime cortisol in infancy.

The genetic and environmental contributions to daytime cortisol vary as a function of the time of the day: moderate to high heritability has been reported at awakening in adults but not later during the day (42). The only gene environment study of daytime cortisol involving an age-homogenous sample of children (12-year-old) revealed a genetic contribution in the morning and early afternoon but not in the evening (34), with the morning samples showing the highest heritability. Thus, there seems to be a gradual circadian shift from genetic to environmental control. However, this environmental contribution does not seem to be experienced similarly by children of the same family (34,43), which points to possible genetic and environmental (G × E) interactions.

Gene–environment interplay has been reported for various health-related phenotypes and with different approaches (44–

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47), including variations in genetic and environmental etiology according to environmental circumstances (48,49). At least two forms of gene-environment interplay might be anticipated. A first possibility, often defined as the "diathesis-stress" model (50), posits that genes that increase vulnerability (or resilience) to stress are more likely to be expressed under adverse/stressful environments than under more favorable conditions (29,51–54). Results showing higher heritability of daytime cortisol secretion under stressful conditions, such as high versus low familial adversity (FA), would be consistent with such a model.

Early adverse environments might also constrain genetic expression. In rodents, early maternal care has long-lasting effect on the HPA axis response to stress (55,56). Findings showing a reduced genetic contribution to cortisol among infants exposed to stressful conditions, such as high versus low FA, would be consistent with that model.

The goal of the present study was to estimate the genetic and environmental contributions to morning cortisol secretion among 6-month-old twins and to investigate whether and how these contributions varied as a function of FA.

Methods and Materials

Participants

Participants were twins recruited between April 1995 and December 1998 in the greater Montréal area to participate in a longitudinal study. A total of 989 families were contacted, of which 672 agreed to participate (68%). Twins were first seen when they were 6 months of gestational age and then prospectively assessed on a variety of child and family characteristics. Informed consent was obtained from the parents annually. Interviews regarding environmental variables were generally conducted with the mother (99.7%). Hospital records were used to get information about pregnancy and delivery. Zygosity was determined through the Zygosity Questionnaire for Young Twins when they were 6 and 19 months of age (57). The DNA-based zygosity was determined for 31% of randomly selected same-sex twin pairs with 8–10 highly polymorphic micro-satellite markers. The two methods yielded a concordance of 93.8% (58).

Saliva samples were collected for 523 children when they were 6 months of age (mean [SD], 5.63 months [.93]). Three twin pairs were excluded, because they were born very premature (26–29 gestational weeks) and with a very low birth weight (≤1000 g), two conditions associated with disturbed HPA axis activity (59,60). The final sample was composed of 517 infants who participated in cortisol sampling at least once (n = 478 and n = 393 for awakening and morning, respectively). Non-genetic statistical analyses (e.g., analyses of variance) were performed with all available twins, but only complete twin pairs were considered for genetic analyses, leaving 232 twin pairs (101 monozygotic [MZ] and 131 dizygotic [DZ] pairs) and 192 twin pairs (67 MZ and 125 DZ pairs) for the awakening and morning samples, respectively. Cortisol levels of infants from complete pairs did not differ from cortisol levels of singleton twins [$t(248) = -1.62$, $p = .11$, and $t(201) = -1.45$, $p = .15$].

Procedures and Measures

Saliva Collection. Two saliva samples were collected 1 week apart: 1) "home-awakening" and 2) "lab in the morning." The "lab in the morning" samples were collected first, immediately upon arrival at the laboratory (approximately 15 min, between 8:32 AM and 10:02 AM; mean [SD], 8:55 [0:14]) with salivettes (Sarstedt Canada, St-Laurent, Québec). Parents were instructed to collect the saliva at home 7 days later, as soon as the child naturally awakes and when he or she is still lying in the bed, unfed. Families were reminded by phone to do so the day before the sampling. Parents were told to put the salivettes in their freezer until the home visit scheduled the following week. The salivettes were brought back to the laboratory and stored at −80°C until assay. The "home-awakening" samples were all collected between 6:00 AM and 10:00 AM (mean [SD], 7:29 AM [0:56]). Mothers were instructed not to feed or give the child anything to drink 20 min before each sampling. All samples were analyzed in a single batch with RadioImmunoAssay (Diagnostic Systems Laboratories, Webster, Texas). The technician was blind as to the zygosity and FA status of the samples. Intra-assay variability was < 10%. Cortisol levels were positively skewed and were normalized with a Log10 transformation (61).

FA

The cumulated risk of FA was assessed by seven perinatal and postnatal risk factors (27): maternal smoking during pregnancy, low birth weight, low family income, low maternal education, single parenthood, young motherhood, and maternal hostile-reactive behaviors. A risk factor was scored if the mother smoked cigarettes across all trimesters (24.9% of the families), birth weight was lower than 2500 g (46.5%), family income was below CDN $20,000 (19.2%), the mother had not completed high school (19.0%), the twins were not living with both biological parents (5.5%), and the mother was younger than 20 years when the twins were born (3.2%). A seven-item, 10-point-Likert (0 = "not at all" to 10 = "exactly") self-report scale was used to assess the mother's hostile-reactive parenting toward each twin (e.g., "I have shaken my baby when he/she was particularly fussy") (Cronbach α = .73) (31). The mother's hostile-reactive scores were strongly correlated across twins of the same family (r_{MZ} = .84, p = .00; r_{MZ} = .78, p = .00) and were thus averaged within families. A risk was counted if the score was above the median.

The resulting FA index was distributed as follows: an FA of 0: 19.2%; 1: 30.5%; 2: 29.0%; 3: 15.0%; 4: 3.5%; 5: 1.6%; 6: 1.2%; and no FA of 7. Families with an FA score of 3 or above were considered to have high levels of FA (21.3%), and those who scored below 3 were considered to have low levels of FA (78.7%). This partition identified an FA risk group that was prevalent enough to conduct meaningful statistical analyses.

Data Analyses

Because having two twins per family implies non-independent observations, differences between groups were tested with two-level hierarchical mixed models (62), with both fixed and random components allowing an unbiased test of difference in means (63,64). Phenotypic associations were tested with intraclass correlations, controlling for zygosity (65), through MPlus (66).

Genetic Modeling

The twin design compares the phenotypic similarity of MZ twins of the same family (100% genetically related) with that of DZ twins of the same family (approximately 50% genetically related) and decomposes the phenotypic variance into three components: additive genetic variance (heritability), shared (common) environmental variance, and non-shared (unique) environmental variance (67). Genetic sources of variance are implied when MZ twins are more similar than DZ twins. Shared environment is indicated when both MZ and DZ pairs are significantly similar; it refers to environmental factors that make twins of the same family similar to each other (e.g., socioeconomic

status, parental mental health, neighborhood). Non-shared environment refers to differences among twins of the same family. That is, experiences that make twins of the same family grow apart (e.g., differences in parenting behaviors, accidents, children's differing peer experiences).

Genetic (A), shared environmental (C), and unique environmental (E) contributions were estimated through structural equation modeling of variance and covariance patterns among MZ and DZ twin pairs, with the MX software (68). Within-pair covariance resulting from the additive genetic effect was posited at 100% for MZ pair and at 50% for DZ pairs, and shared environment was posited at 100% for both MZ and DZ twin pairs. Unique environment was estimated as residual variance and included measurement error. All twin pairs were concordant for FA. Models that allowed A, C, and E parameters to vary according to FA were compared with models that constrained parameters to be equal across FA groups. The full ACE models were tested against simpler, nested models (e.g., AE, CE, E models). Best models were selected according to goodness of fit (χ^2 test) and parsimony indices such as Akaike Information Criteria (AIC) and the Root Mean Squared Error of Approximation (RMSEA). Cortisol was examined for outliers, defined as scores of ± 3 SD from the mean (69,70); none were found.

Table 1. "Home-Awakening" and "Morning in the Lab" Cortisol Descriptive Statistics According to a Variety of Pre-, Peri- and Postnatal Variables

	Awakening (Day 1)			Morning (Day 2)		
Variables	Mean	SD	*n*	Mean	SD	*n*
Zygosity						
Monozygotes	.58	.37	205	.45	.37	137
Dizygotes	.57	.45	273	.41	.30	256
Gender						
Male Subjects	.54	.42	224	.43	.31	153
Female Subjects	.61	.41	254	.42	.34	240
Ethnicity						
Caucasians	.58	.43	412	.44	.34	335
Others	.58	.39	59	.39	.26	54
Birth Weight						
≥2500 g	.55	.34	245	.43	.33	192
<2500 g	.60	.44	203	.41	.30	180
Gestational Age at Birth						
≥37 weeks	.55	.36	195	.43	.34	183
<37 weeks	.59	.42	229	.41	.30	163
Gestational Diabetes						
Yes	.51	.39	62	.43	.24	52
No	.57	.39	324	.42	.33	316
Gestational Hypertension						
Yes	.50	.38	68	.39	.26	62
No	.57	.39	334	.43	.33	308
Expected Pregnancy						
Yes	.57	.40	294	.42	.32	250
No	.52	.36	110	.42	.31	120
Postnatal Depression						
Yes	.58	.40	88	.39	.33	75
No	.55	.38	312	.43	.32	300
Instrumentation Used for Delivery						
None	.60	.37	173	.44	.31	158
Forceps	.44	.31	15	.30	.16	11
Suction-grip	.53	.54	20	.39	.29	16

Raw data (μg/dL). No significant differences were found at $p < .05$.

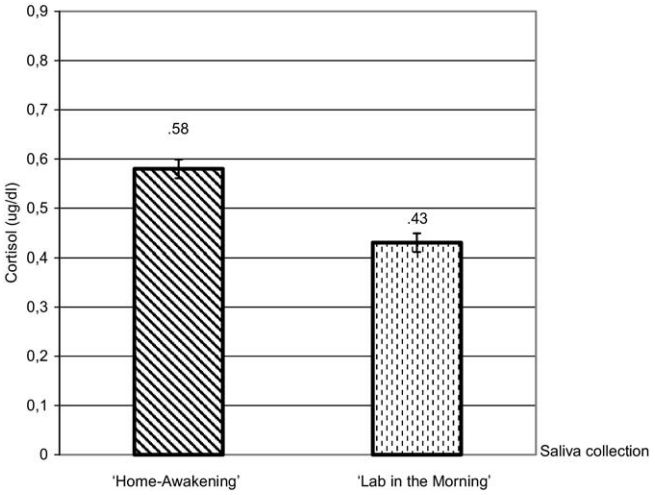

Figure 1. Mean and SEMs of cortisol levels at awakening and morning for all twins ($n = 478$ and $n = 393$ for the awakening and morning samples, respectively).

Results

Table 1 presents the descriptive statistics of daytime cortisol samples according to pre-, peri- and postnatal variables. No significant differences were found at $p < .05$. Awakening cortisol was lower when sampled later [$t(191) = -2.92$, $p = .003$], perhaps because sleep routine often differs across infants, and parents were instructed to sample saliva when the child naturally awakes. No such difference was detected for the morning sample [$t(201) = 1.31$, $p = .19$]. The time of saliva collection did not vary according to FA status [$t(193) = 1.39$, $p = .17$; and $t(262) = -.26$, $p = .79$] and zygosity [$t(193) = -.11$, $p = .91$; and $t(262) = .88$, $p = .38$].

Means and SEMs of cortisol samples are illustrated in Figure 1. Consistent with the circadian rhythm, cortisol level was higher at awakening than in the morning [β (SEM) = −.14 (.03), $p < .001$]. This difference did not vary according to FA [β (SEM) = −.05 (.05), $p = .35$]. The correlation between the samples did not reach significance [$r = .07$, $p = .19$].

FA and Cortisol Secretion

No mean differences were found between twins exposed to low versus high FA for both samples [$t(248) = -1.04$, $p = .30$; and $t(201) = .15$, $p = .88$]. We also partitioned the samples into quartiles, allowing for different associations to emerge at the lower (1st quartile) or higher (4th quartile) ends of the distribution. No significant association was found (polychoric correlations: ρ = −.11, $p = .15$; and ρ = −.04, $p = .65$).

MZ and DZ Intraclass Correlations

Figure 2 presents the MZ and DZ intraclass correlation coefficients (ICC) for all twins and according to FA. For all twins, the MZ-DZ ICC discrepancy seemed larger for "home-awakening" cortisol than for "lab in the morning" cortisol, suggesting a larger contribution of genetic factors earlier in the day.

A differentiated pattern of MZ-DZ ICCs emerged according to FA. For the "home-awakening" cortisol, MZ ICCs tended to be higher than DZ ICCs in both FA settings, although the MZ-DZ difference seemed attenuated in high FA. A different pattern was found for "lab in the morning" cortisol: in low FA, both MZ and DZ ICCs were low, whereas in high FA, a large MZ-DZ discrepancy was found, pointing to a higher genetic contribution to

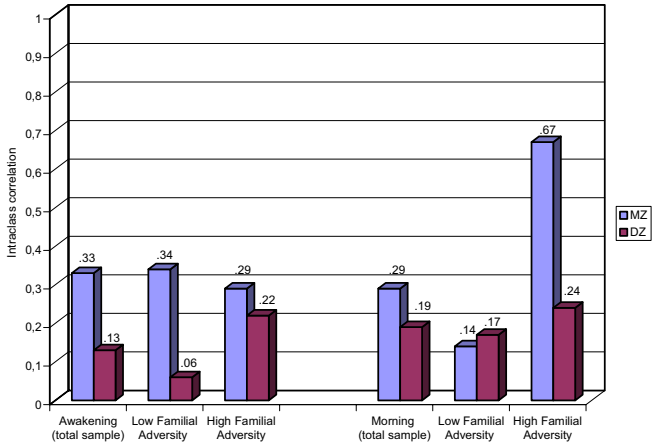

Figure 2. Intraclass monozygotic (MZ) and dizygotic (DZ) correlations for "home-awakening" and "morning in the lab" cortisol samples for all twins and according to familial adversity. Awakening: all twins: MZ (n = 101 pairs); DZ (n = 131 pairs); Low Familial Adversity: MZ (n = 77 pairs); DZ (n = 101 pairs); High Familial Adversity: MZ (n = 24 pairs); DZ (n = 30 pairs). Morning: all twins: MZ (n = 67 pairs); DZ (n = 125 pairs); Low Familial Adversity: MZ (n = 47 pairs); DZ (n = 95 pairs); High Familial Adversity: MZ (n = 20 pairs); DZ (n = 30 pairs).

cortisol secretion in high FA than in low FA (scatterplots available in Supplement 1). Finally, ICCs were also calculated with more liberal (≥2 risk factors) and restrictive (≥4 risk factors) criteria. Similar patterns of results were obtained (figures available on request).

Genetic and Environmental Contributions to Daytime Cortisol Samples

Home-Awakening. Model-fitting results for "home-awakening" cortisol are presented in Table 2. We examined models testing the invariance of the genetic and the environmental parameters across FA groups (i.e., constraining the parameters to be equal [EQ] across FA groups). From the full ACE, the elimination of the A or C parameters did not worsen the fit of the model [$\Delta\chi^2(1) = 3.32$, $p = .07$; and $\Delta\chi^2(1) = .00$, $p = >.99$], whereas including only the E parameter did [$\chi^2(2) = 11.56$, $p = .003$]. The AE and CE models were thus more parsimonious than the ACE model, with the AE model performing better according to the AIC. We then allowed parameters to differ across FA settings (nonequal [NEQ] models). From the ACE-NEQ full model, eliminating successively the C for both low FA (model 6) and high FA groups (model 7) did not deteriorate the model [$\Delta\chi^2(2) = .00$, $p = >.99$]. Then, eliminating the A for either the low FA (model 8) or the high FA (model 9) led to worse models [$\Delta\chi^2(1) = 7.49$, $p = <.01$; and $\Delta\chi^2(1) = 13.81$, $p = <.001$].

Models 2, 3, and 7 were kept according to their goodness of fit (χ^2). The AE-EQ model (model 2) was selected, because it offered the best balance between explanatory power and parsimony (AIC = −10.434 and RMSEA = .058): "home-awakening" cortisol was accounted for by genetic (A = .32) and non-shared environmental (E = .68) factors, and this pattern did not vary as a function of FA.

Lab in the Morning

The model-fitting results for the "lab in the morning" sample are presented in Table 3. From the ACE-EQ full model, reducing to AE or CE did not significantly worsen the fit [$\Delta\chi^2(1) = .24$, $p = .62$; and $\Delta\chi^2(1) = .40$, $p = .53$], whereas only inclusion of the E did [$\Delta\chi^2(2) = 9.84$, $p = <.01$]. From the ACE-NEQ full model (model 5), removing successively the C in high FA (model 6), the A in low FA (model 7), and the C in low FA (model 8) did not lead to a worse fit [$\Delta\chi^2(1) = .72$, $p = .40$; $\Delta\chi^2(1) = .00$, $p = >.99$; and $\Delta\chi^2(1) = 3.42$, $p = .06$]. However, eliminating the A for the high FA (model 8) weakened the fit [$\Delta\chi^2(1) = 9.75$, $p = <.01$].

Table 2. Summary of the ACE Model-Fitting Results of the "Home-Awakening" Cortisol Sample According to FA

	Fit Statistics						Estimated Components (95% CI)		
Models	χ^2	*df*	*p*	AIC	RMSEA		A	C	E
				Equal Models					
1. ACE	9.57	9	.39	−8.434	.063	LFA	.32 (.00–.51)	.00 (.00–.24)	.68 (.53–.88)
						HFA	.32 (.00–.51)	.00 (.00–.24)	.68 (.53–.88)
2. AE[a]	9.57	10	.48	−10.434	.058	LFA	.32 (.13–.51)	—	.68 (.53–.88)
						HFA	.32 (.13–.51)	—	.68 (.53–.88)
3. CE	12.89	10	.23	−7.115	.076	LFA	—	.19 (.06–.33)	.81 (.68–.98)
						HFA	—	.19 (.06–.33)	.81 (.68–.98)
4. E	21.13	11	.03	−.871	.129	LFA	—	—	1.00 (1.0–1.0)
						HFA	—	—	1.00 (1.0–1.0)
				Non-Equal Models					
5. $A_1C_1E_1$ $A_2C_2E_2$	5.67	6	.46	−6.334	.049	LFA	.28 (.00–.50)	.00 (.00–.00)	.72 (.55–.95)
						HFA	.43 (.00–.81)	.00 (.00–.53)	.57 (.33–.81)
6. A_1E_1 $A_2C_2E_2$	5.67	7	.58	−8.334	.041	LFA	.28 (.08–.50)	—	.72 (.55–.95)
						HFA	.43 (.00–.81)	.00 (.00–.53)	.57 (.33–.81)
7. A_1E_1 A_2E_2	5.67	8	.69	−10.334	.037	LFA	.28 (.08–.50)	—	.72 (.55–.95)
						HFA	.43 (.06–.81)	—	.57 (.33–.81)
8. E_1 A_2E_2	13.16	9	.16	−4.843	.075	LFA	—	—	1.00 (1.0–1.0)
						HFA	.43 (.06–.81)	—	.57 (.33–.81)
9. A_1E_1 E_2	19.48	9	.02	1.48	.150	LFA	.00 (.00–.00)	—	1.00 (.45–1.0)
						HFA	—	—	1.00 (.88–1.0)

For the Non-Equal Models, the parameters of the Low Familial Adversity group (LFA) are indicated as 1, whereas 2 refers to High Familial Adversity group (HFA) parameters. A, Genetic estimate; C, shared environment estimate; E, unique environment estimate; FA, familial adversity; CI, confidence interval; AIC, Akaike Information Criteria; RMSEA, Root Mean Squared Error of Approximation; MZ, monozygotic twins; DZ, dizygotic twins.

[a]Selected best-fitting model.

Table 3. Summary of the ACE Model-Fitting Results of the "Morning in the Lab" Cortisol Sample According to FA

Fit Statistics						Estimated Components (95% CI)			
Models	χ^2	*df*	*p*	AIC	RMSEA		A	C	E
Equal Models									
1. ACE	9.65	9	.38	−8.354	.071	LFA	.18 (.00–.51)	.10 (.00–.37)	.72 (.53–.95)
						HFA	.18 (.00–.51)	.10 (.00–.37)	.72 (.53–.95)
2. AE	9.89	10	.45	−10.110	.060	LFA	.31 (.12–.51)	—	.69 (.53–.91)
						HFA	.31 (.12–.51)	—	.69 (.53–.91)
3. CE	10.05	10	.44	−9.949	.076	LFA	—	.22 (.08–.38)	.78 (.64–.96)
						HFA	—	.22 (.08–.38)	.78 (.64–.96)
4. E	19.49	11	.05	−2.509	.138	LFA	—	—	1.00 (1.0–1.0)
						HFA	—	—	1.00 (1.0–1.0)
Non-Equal Models									
5. $A_1C_1E_1$ $A_2C_2E_2$	3.60	6	.73	−8.399	.013	LFA	.19 (.00–.43)	.00 (.00–.36)	.81 (.60–.10)
						HFA	.69 (.01–.89)	.00 (.00–.00)	.31 (.17–.62)
6. $A_1C_1E_1$ A_2E_2	2.88	7	.90	−11.123	.000	LFA	.00 (.00–.40)	.16 (.00–.34)	.84 (.67–.10)
						HFA	.69 (.33–.89)	—	.31 (.17–.62)
7. C_1E_1 A_2E_2	2.88	8	.94	−13.123	.000	LFA	—	.16 (.00–.34)	.84 (.67–1.0)
						HFA	.69 (.33–.89)	—	.31 (.17–.62)
8. E_1 A_2E_2[a]	6.30	9	.71	−11.698	.023	LFA	—	—	1.00 (1.0–1.0)
						HFA	.69 (.33–.89)	—	.31 (.17–.62)
9. C_1E_1 E_2	16.05	9	.07	−1.952	.146	LFA	—	.16 (.00–.34)	.84 (.67–1.0)
						HFA	—	—	1.00 (1.0–1.0)

For the Non-Equal Models, the parameters of the LFA are indicated as 1, whereas 2 refers to HFA parameters. Abbreviations as in Table 2.
[a]Selected best-fitting model.

Models 2, 3, and 8 were retained according to their goodness of fit. Model 8 clearly offered the best balance between explanatory power and parsimony (AIC = −11.698, and RMSEA = .023) and was thus retained. In low FA, "lab in the morning" cortisol was accounted for by unique environment, whereas in high FA, both genetic (A = .69) and unique environment (E = .31) factors contributed to the phenotype (this genetic-environmental etiology did not vary as a function of gender in both samples [not shown]).

Discussion

The goal of this study was to examine the genetic and environmental contributions to morning cortisol secretion in 6-month-old twins and to determine whether these contributions varied according to FA. Genetic factors accounted for cortisol levels in different ways: a moderate "main effect" of genes was found for home-based awakening cortisol, whereas the contribution of genes to morning cortisol in the laboratory was conditional to FA. Specifically, in low FA settings (typical of most families), there was no genetic contribution and only unique environmental contributions to lab-based morning cortisol. In high FA, lab-based morning cortisol was mainly accounted for by genetic factors and, to a lesser extent, by unique environmental factors.

Whereas the finding of a genetic "main effect" for awakening cortisol at home is consistent with results from other studies (34,42,43), the differential pattern of genetic and environmental etiology of laboratory-based morning cortisol according to FA is a new and striking feature of the present results. Overall, these findings suggest a complex and evolving process of G×E interactions underlying early daytime cortisol. A number of points should be underlined.

First, the differential pattern of genetic and environmental contributions to laboratory-based morning cortisol as a function of FA is consistent with a "diathesis-stress" model suggesting that the genetic liability (or resilience) to stress is more likely to be expressed in adverse environments than in more favorable conditions. This is the first study to reveal a conditional contribution of genes according to FA at such an early age. Specific genetic liability could modulate the impact of early adversity on cortisol activity and, more generally, the vulnerability to stress-related diseases later in life (71). These genetic liabilities could be expressed at several sites involved in the regulation of cortisol activity, including upstream structures that modulate the HPA axis. For example, increased activation of the right amygdala in response to fearful stimuli has been noted in the short allele serotonin transporter (5HTT) gene carriers located in the promoter region (72). Future studies should thus examine the potential role of measured genes, such as the short 5HTT allele, in the present conditional association.

Second, the strong genetic contribution to morning cortisol levels in high FA runs opposite to the idea of a programming effect of early adversity on cortisol activity at 6 months of age. Robust programming and epigenetic effects of maternal care have been reported in rodents during the stress hyporesponsive period (73–75). The dampened HPA axis response to stress and low circulating basal cortisol levels typical of this period could protect the immature but fast developing brain from repeated and prolonged glucocorticoid exposure (see 76). To the extent that a functionally equivalent period emerges in the end of the first year of life in humans, it might be too early at 6 months of age for FA to have a programming effect on daytime cortisol activity (one major difference is that postnatal adversity is more likely to affect corticolimbic structures and pathways than the HPA axis per se given the relative maturity of this system at birth in primates [77]; accordingly, adversity experienced during this period could influence corticolimbic structures and circuitry, which in turn, could affect cortisol reactivity and regulation; an important buffering role of the caregiver is also presumed [25,40,74,78–81]).

Such a programming effect of FA was suggested in a recently

reported pattern of genetic and environmental contributions to cortisol response to novelty in 19-month-old twins (82). Specifically, both shared and unique environments, not genetic factors, accounted for cortisol reactivity in high FA settings. In contrast, under low FA settings, only genetic and uniquely experienced factors contributed to the phenotype. The divergent patterns found in the two studies could be due to the period of development (6 vs. 19 months) or to the nature of HPA activity (daytime cortisol secretion vs. response to social novelty). Clearly, additional genetically informative studies collecting multiple daytime cortisol samples longitudinally in early childhood are needed to understand the role of environmental adversity in HPA activity in humans.

Third, the moderation of a genetic contribution to cortisol activity by FA was restricted to the morning cortisol sample in the laboratory and did not affect awakening cortisol levels at home. A moderate heritability of awakening cortisol has been reported in previous twin studies (39,42,43), although not as early in development. Why was genetic contribution to cortisol conditional to FA for morning cortisol but not for awakening cortisol? One possibility is that cortisol activity might be regulated by different structures at awakening versus later during the morning (42). Specifically, the suprachiasmatic nucleus progressively reduces its inhibitory control on the hypothalamus in the second half of the night, resulting in an overall increase in cortisol secretion. Approximately 2 hours before the awakening, neurons located in the paraventricular nucleus of the hypothalamus enhance its vasopressin secretion for a short period of time, which temporarily boost cortisol secretion at awakening before decreasing drastically during the next hour (83,84). These basic physiological functions could be more attuned to genetic variation. In contrast, cortisol secretion taking place later during the morning is intended to facilitate adaptation to changing environments through cognitive, attention, and emotion regulation processes and thus could be more sensitive to environments.

Fourth, in contrast to previous singleton studies (40,85), morning cortisol levels did not differ as a function of FA. Many factors could account for this finding. First, as suggested by the difference in heritability in lab-based morning cortisol according to FA, there could be a difference in morning cortisol levels only for infants carrying a genetic risk (i.e., a G×E interaction). Second, differences in morning cortisol as a function of FA might emerge with age, possibly as a result of chronic exposure to adversity (86). Finally, at 6 months of age, cortisol levels might vary according to FA only later during the morning. The increased environmental control of afternoon cortisol levels in comparison with morning cortisol is consistent with this idea (42,43,80).

The present findings have important clinical implications. The genetic modulation of cortisol activity by FA might point to one mechanism through which adverse life conditions might exacerbate individual liabilities (or resilience) to stress and stress-related diseases (87,88). This finding stresses the importance of identifying the genetic variants involved and of characterizing the underlying mechanisms to help policymakers and clinicians to implement early preventive interventions tailored for children at risk for atypical patterns of cortisol.

Limitations of the Study

A few features of the current study might have constrained the findings. First, the cortisol indices were each based on a single saliva sample. Multiple samples collected over several days would have yielded more reliable measures. However, despite this limitation, significant heritability estimates were revealed, and different patterns of genetic and environmental contributions were found. Second, morning cortisol samples were collected in a non-familiar context (laboratory visit) that might have been arousing for some children. It is thus possible that it partly reflected reactive cortisol. However, awakening and morning mean cortisol levels showed the expected circadian rhythm, suggesting that the visit did not induce a stress response in most children. Third, the study would have benefited from a more extended coverage of the day to examine whether distinct patterns of genetic and environmental contributions as a function of FA would have been found. Fourth, the sample presented a limited number of cases of severe FA. Whether the findings generalize to the far end of the spectrum of FA is open to question. Fifth, the assessment of maternal hostile/reactive behaviors relied on self-report and might have been influenced by social desirability despite the use of contextualized items (i.e., the child's difficult behavior) (31,89). Finally, because the FA index was computed as a family-level variable, it was not possible to establish whether the effect was genuinely environmental or genetically mediated.

Conclusions

The present study was the first to reveal distinct patterns of genetic and environmental contributions to morning cortisol secretion in the laboratory according to adversity in the first year of life. Genetic factors accounted for most of the variance in morning cortisol in high FA but not in low FA, suggesting the presence of a "diathesis" more likely to be expressed in adverse settings. Clearly, these conditional contributions of adversity and genetic factors need to be replicated in genetically informative studies with larger samples and with respect to the nature, timing, duration, and intensity of the adversity (24,86,90–92). Finally, future studies should investigate to what extent daytime cortisol early in development predicts later vulnerability to stress.

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Supplementary material cited in this article is available online.

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