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High cortisol levels in the offspring of parents with bipolar disorder during two weeks of daily sampling

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Original Article

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Objectives: The hypothalamic-pituitary-adrenal (HPA) axis is compromised in major depression, bipolar disorder (BD), and in the offspring of parents with major depression. Less is known about the offspring of parents with BD (FH+). The present project provides follow-up to a previous study showing that the adolescent (mean age 16.7 years) FH+ offspring had higher salivary cortisol levels than the offspring of parents with no mental disorder (FH-) throughout the day in their natural environment, and that girls had higher cortisol levels than boys (Ellenbogen MA, Hodgins S, Walker C-D, Adam S, Couture S. Daytime cortisol and stress reactivity in the offspring of parents with bipolar disorder. *Psychoneuroendocrinology* 2006; 31: 1164–1180). The goal of the present study was to determine whether FH+ offspring, approximately two years later, continued to exhibit elevated cortisol levels relative to FH- offspring during two weeks of daily sampling.

Methods: The present study examined salivary cortisol levels in 24 (18.3 ± 2.6 years) FH+ and 22 (18.0 ± 2.3 years) FH- offspring who are part of the same longitudinal cohort as the previous study. Saliva was collected at 1300h and 1500h in the natural environment of the offspring during 14 consecutive days.

Results: Multilevel modelling analyses indicated that FH+ offspring had higher afternoon levels of cortisol in their natural environment than FH- offspring, but group differences in slope and gender differences were not found.

Conclusions: The FH+ offspring exhibited increased daytime secretion of cortisol that, at the level of the group, persisted into late adolescence and young adulthood. Perhaps this change in HPA functioning is associated with an increased vulnerability for the development of an affective disorder.

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Converging evidence indicates that the hypothalamic-pituitary-adrenal (HPA) axis is severely compromised in major depression and bipolar disorder (BD) (1–3), some of which persists into

remission (1, 4). Among the multiple HPA dysfunctions associated with the affective disorders (5), elevated patterns of cortisol secretion during the night and following awakening have been observed in patients with BD (6, 7) and major depression (4, 8).

Studies of cortisol levels in adults and children at risk for major depression have reported similar results. Parental depression has been associated

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with elevated cortisol levels among their healthy offspring in studies of infants, children, and young adults (9–12). In a prospective study of high-risk adolescents, elevated morning cortisol during one of four sampling days was predictive of the development of major depression during the following 12 months (13). Risk status in youth was defined by high emotionality, exposure to multiple negative life events or marital discord, or having a parent with a psychiatric disorder, all of which could also be indicators of exposure to chronic stress. In a prospective study of offspring of parents with major depression, elevated morning cortisol levels at age 13 were associated with maternal postnatal depression (11). Importantly, elevations in cortisol at age 13 were predictive of depressive symptoms, but not the clinical diagnosis, at age 16 (14).

Studies of HPA functioning in the offspring of parents with BD [family history positive (FH+)], who are at high risk for developing an affective disorder (15), are sparse, and the results have been mixed. A study of daytime salivary cortisol levels and another examining the cortisol response to corticotropin-releasing hormone (CRH) have found no robust group differences (16, 17). In contrast, we reported that at the mean age of 16.7 years, the FH+ offspring had higher salivary cortisol levels following awakening and during the day than the offspring of parents having no mental disorder [family history negative (FH–)] (18, 19). Moreover, girls had higher cortisol during the day than boys, and this effect was independent of risk status. Differences between the high- and low-risk adolescents in daytime cortisol levels were not related to the small number of participants with diagnoses of mental disorders, nor were they associated with self- or parent-reports of clinical symptoms. The findings of the latter study and those of others (14) suggest that elevated cortisol levels in participants at high risk for developing an affective disorder may represent a putative pre-morbid marker of vulnerability.

The present study was undertaken to follow up the previous findings from our group (18) in FH+ and FH– offspring. Because sustained elevations of glucocorticoids are detrimental to human health but time-limited elevations are adaptive (20), it is important to determine whether elevations in cortisol observed previously (at the mean age of 16.7 years) persist over time. Moreover, the present study examined salivary cortisol levels over a longer sampling period than our previous study. An extended period of sampling increases the likelihood of identifying meaningful correlates and outcomes associated with salivary cortisol

levels (14, 21). Thus, in the present study, salivary cortisol levels were examined at 1300h and 1500h for 14 consecutive days in the FH+ offspring and controls. These sampling times were chosen because group differences were greatest during the afternoon in the previous study (18). Participants, now in late adolescence or early adulthood (mean age of 18 years), were selected from an ongoing longitudinal study. Many (but not all) had completed the previous cortisol assessment two to three years earlier. It was hypothesized that the FH+ offspring would exhibit higher afternoon cortisol levels, independent of mental health status, than the FH– offspring. In contrast, it was predicted that the slope, or rate of decline, during the afternoon would be similar between groups. As a secondary goal, we also compared groups on evening cortisol levels and the cortisol increase following awakening, based on a two-day sampling protocol.

Although we previously found a robust sex difference in cortisol levels among our adolescent sample (18), we did not expect to replicate this finding. Sex differences in basal cortisol levels, although evident in adolescence (18, 22), have not been observed in adult samples (23). Finally, the relationship between self-reported stress, anxiety, depression, and externalizing problems will be examined both as predictors of salivary cortisol levels and as possible moderators of the relationship between risk status and cortisol.

Methods

Participants

Twenty-four FH+ offspring from 18 families and 22 FH– offspring from 16 families were randomly selected from a subject pool of 189 15–25 year olds participating in a longitudinal study of FH+ and FH– offspring (24). Parents with BD and their spouses were originally recruited from general hospitals and advocacy and support groups in the Canadian province of Quebec. Parents with no mental disorder were selected from the same geographical regions as parents with BD. Parental diagnoses (or the absence thereof) were confirmed by an experienced clinician using the Structured Clinical Interview for DSM-IV (25) and from an examination of psychiatric records. Out of 64 offspring candidates contacted for the present study (26), 3 high-risk offspring and 11 low-risk offspring refused to participate, and 4 offspring (2 FH+ and 2 FH–) were noncompliant in the collection of saliva. Among those who participated, 20 offspring had a parent (10 mothers,

8 fathers) diagnosed with bipolar I disorder, and 4 offspring had a parent (2 mothers, 2 fathers) diagnosed with bipolar II disorder. Thirty-two subjects (16 FH+ and 16 FH-) had participated in the previous study of salivary cortisol levels two to three years earlier (18).

All participants were administered the Structured Clinical Interview for DSM-IV, Patient Edition (25), by clinical psychologists. Demographic and clinical information are presented by group in Table 1. FH+ youth ($n = 6$; 25%) were more likely to have a lifetime affective disorder than FH- youth ($n = 2$; 9%), but the difference did not achieve statistical significance [$\chi^2 (1, N = 46) = 2.2$, NS]. Nevertheless, the presence of a lifetime affective disorder was statistically controlled for in the data analyses. Participants were in good physical health, except that one participant (FH+) reported having asthma, and another (FH+) reported hypertension. These participants were being treated, respectively, with salbutamol (Ventolin) and lisinopril (Zestril). Other medications being used by participants were selective serotonin reuptake inhibitors ($n = 2$, FH+), finasteride (Propecia; $n = 1$, FH+), mometasone nasal spray (Nasonex; $n = 1$, FH+), and unspecified acne medications ($n = 1$, FH+; $n = 2$, FH-).

Questionnaires

Offspring who were 19 years or older ($n = 17$) completed the Beck Depression Inventory (27) and

the Penn State Worry Questionnaire (PSWQ) for Adults (28), and those who were 18 years or younger ($n = 30$) completed the Child Depression Inventory (29) and the PSWQ for Children (30). All participants also completed the Youth Self-Report version of the Child Behavior Checklist (31), which assesses internalizing and externalizing behaviors, and the High School Students' Recent Life Experiences Questionnaire (32), a measure of daily hassles among youth.

Procedure

Following telephone contact, participants provided written consent to participate in the study, completed the questionnaires, and underwent a diagnostic interview by an experienced clinician during a laboratory visit. For participants 17 years or younger, parents also provided written consent.

Participants were provided with detailed oral and written instructions for collecting saliva at home. They collected saliva at 1300h and 1500h for 14 consecutive days while following their routine schedule. Additional morning (awakening, awakening + 30 min, and awakening + 60 min) and evening (2000h and bedtime) samples were collected during the first two days of the two-week protocol. Participants were instructed to remove lipstick, to refrain from drinking water at least five minutes before sampling, and to refrain from eating, drinking (except water), smoking, and brushing teeth at least 60 min before sampling. Participants recorded the time they took the

Table 1. Clinical and demographic information among the offspring of parents with bipolar disorder (BD) and parents with no mental disorder (NMD)

	Offspring of BD parent ($n = 24$)	Offspring of NMD parent ($n = 22$)
Age, mean (SD) [range]	18.3 (2.6) [15–25]	18.0 (2.3) [15–24]
Gender	12F/12M	11F/11M
Lifetime diagnosis, n (%)	7 (29)	2 (9)
Bipolar disorder	3	0
Major depressive disorder	3	2
Substance use disorder	1	0
Currently symptomatic, n (%)	1 (4)	1 (5)
Bipolar disorder	1	0
Major depressive disorder	0	1
Substance use disorder	0	0
Depression (z-score), mean (SD) ^a	-0.04 (0.83)	0.03 (1.1)
Anxiety (z-score), mean (SD) ^a	0.13 (0.89)	-0.16 (1.1)
Daily hassles, mean (SD) ^b	75.3 (19.1)	70.4 (14.0)
Internalizing problems, mean (SD) ^c	45.8 (5.6)	47.8 (8.5)
Externalizing problems, mean (SD) ^c	52.2 (9.7) ^d	46.7(8.1)
Psychotropic medication, n (%)	2 (8)	0 (0)

^az-scores were used here because different symptom measures were used for those below and above 18 years of age.

^b $n = 45$, data missing in one FH- offspring.

^ct-scores; $n = 43$, data missing in one FH+ and two FH- offspring.

^d $p < 0.05$, FH+ versus FH-.

sample and activities prior to sampling. During the period of 14 days, participants also recorded their mood and behaviour during social interactions, but these are reported elsewhere (26). Participants were remunerated \$100 CAN for their participation.

Saliva sampling

Saliva was absorbed into a small cotton roll and expressed through a plastic tube into a sterile vial [Salivette device (Sarstedt AG & Co., Nümbrecht, Germany)]. Saliva samples were frozen at -20°C until assayed for cortisol by a sensitive radioimmunoassay (33) using a commercial kit from Diagnostic Systems Laboratory, Inc. (DSL-2000; Sanofi Diagnostics, Montréal, Canada). The sensitivity of the assay was set at $0.01\ \mu\text{g}/\text{dL}$ (or $0.276\ \text{nmol}/\text{L}$). The inter- and intra-assay coefficient of variations for all assays were 3.6% and 4.6% (on a range of $0.01\text{--}10\ \mu\text{g}/\text{dL}$ dose), respectively. Assays were conducted in the laboratory of C-DW at the Douglas Hospital Research Centre (Montreal, Canada).

Statistical analyses

To analyze the data in such a way as to identify the variance accounted for by each variable at several levels, a multilevel modeling program (hierarchical linear modeling) was used (34). Multilevel modeling has the distinct advantage with data such as these because it can accommodate for violations of the statistical assumption of independence in sampling. That is, a person's cortisol level at a given time point is inherently dependent on the previous cortisol level and will subsequently influence later cortisol levels. Moreover, multilevel modeling can also accommodate for violations of the assumption of fixed effects. Therefore, the effect of time may be associated with increases in cortisol level in some people and decreases among others. Multilevel modeling allows for the testing of variability in effects and for the inclusion of variables to account for such variability. In the level-1 analysis (within-subject), the individual's cortisol levels were used as the dependent variable, and time was used as a predictor. At level 2 (between-subject), the characteristics of the participants were used to account for variability observed in the level-1 effects. The models were built up gradually, starting from a null model that contained only the distinction between within-subject and between-subject variance, so as to be a reference point for the later models. Once the effect of time was added to the models, between-subject variability was tested to identify whether people

differed in change over time using chi-square tests. Between-subject variables had to significantly influence cortisol levels (intercept) or the change in cortisol over time (slope) while simultaneously and significantly decreasing the amount of between-subject variability (as tested by a chi-square difference test). All probability (p) values for the t -tests detailed below are based on one-tailed tests of significance due to *a priori* expectations of the direction of the effects. The percentages of variance accounted for are based on estimates of variance of the final model compared to that of the previous model.

Results

Number of samples and compliance

The mean number of samples collected during the afternoon protocol was 22.2 ± 6.2 [range 4–28], with 10.8 ± 3.3 [range 3–14] and 11.4 ± 3.2 [range 1–14] provided at 1300h and 1500h, respectively. FH+ and FH– offspring provided 22.3 ± 6.0 [range 11–28] (1300h: 10.9 ± 3.4 ; 1500h: 11.4 ± 2.9) and 21.5 ± 7.0 [range 4–28] (1300h: 10.5 ± 3.5 ; 1500h: 11.0 ± 3.7) samples, respectively. Self-reported compliance ($n = 45$) indicated that, on average, FH+ and FH– youth sampled 11.1 ± 10.9 and 4.3 ± 10.2 min following the 1300h sample, respectively, and 21.6 ± 14.5 and 17.0 ± 13.1 following the 1500h sample, respectively. Mixed-design ANOVAs (group \times sample) of these data indicated that there were no group differences or group \times sample interactions for the number of valid samples, but a trend for significance for the main effect of group was observed for the compliance data [$F(1,43) = 3.9$, $p = 0.054$]. For this reason, the mean time before or after the designated sampling time (in minutes) was added as a covariate in the analyses below.

To determine if self-report compliance declined over time, we compared the mean overall compliance (in minutes) over the last four days (15.1 ± 13.6), the middle five days (15.9 ± 13.9), and the first five days (12.7 ± 13.2). There were no significant differences in compliance over the three periods. Finally, objective compliance data were collected for 14 participants, as part of a pilot study, using vials with time-stamping microcircuitry in the cap [MEMS 6 (TrackCap, Aardex Ltd., Zug, Switzerland)]. Correlations between time-stamping compliance and self-report compliance were 0.70 ($p < 0.01$) and 0.83 ($p < 0.001$) for the 1300h and 1500h samples, respectively. Thus, self-reported compliance to the saliva sampling

Cortisol in high-risk offspring

protocol was consistent with objective time-stamping methods in a small sample of participants.

Afternoon cortisol levels and risk status

Among FH+ and FH- offspring, mean (\pm SD) cortisol levels ($\mu\text{g}/\text{dL}$) averaged over the 14 days were, respectively, 0.179 ± 0.075 and 0.151 ± 0.083 at 1300h, and 0.155 ± 0.074 and 0.130 ± 0.070 at 1500h. The unconditional model revealed that 69.77% of the variance in afternoon cortisol levels was related to within-subject variability, while the remaining amount was attributable to between-subject variability. Time of sampling (1300h or 1500h) was negatively associated with cortisol [$B = -0.012$, $SE = 0.004$, $t(746) = 3.21$, $p < 0.05$], confirming that cortisol decreased significantly over the afternoon. Next, the effect of risk group was added to the between-subject model. Beforehand, sex, age, medications, subjective compliance (minutes before or after the designated sampling time), food consumption (coded as having eaten within 60 min of sampling on any sample), smoking, exercise (coded as having exercised before sampling on any sample), oral contraceptive use, and lifetime diagnosis of major affective disorder were added to the model as covariates. Risk status was significantly associated with an overall increase in cortisol [$B = 0.020$, $SE = 0.011$, $t(34) = 1.85$, $p < 0.05$]. The addition of the risk status variable led to a significant decrease in between-subject variability [$\Delta\chi^2(1) = 34.55$, $p < 0.05$], explaining 4.48% of the between-subject variance. These data are depicted graphically in Fig. 1, where mean cortisol levels by group are presented at each time point. No other between-subject factor was significantly related to cortisol levels. In sum, the analyses confirmed the expected diurnal slope of daytime cortisol levels and indicate that FH+ offspring have higher afternoon cortisol levels than FH- offspring.

Afternoon cortisol levels, risk status, and behavioral predictors

In Table 1, measures of affective symptoms and behaviors are presented by risk group. To determine whether the group difference reported above was influenced by behavioral indicators of risk, the above analyses were repeated using depression, anxiety, internalizing problems, externalizing problems, daily hassles, and their interactions with risk status as between-subject level-2 predictors. To reduce the number of between-subject variables, we excluded the set of covariates in the analyses described above since none of them were statisti-

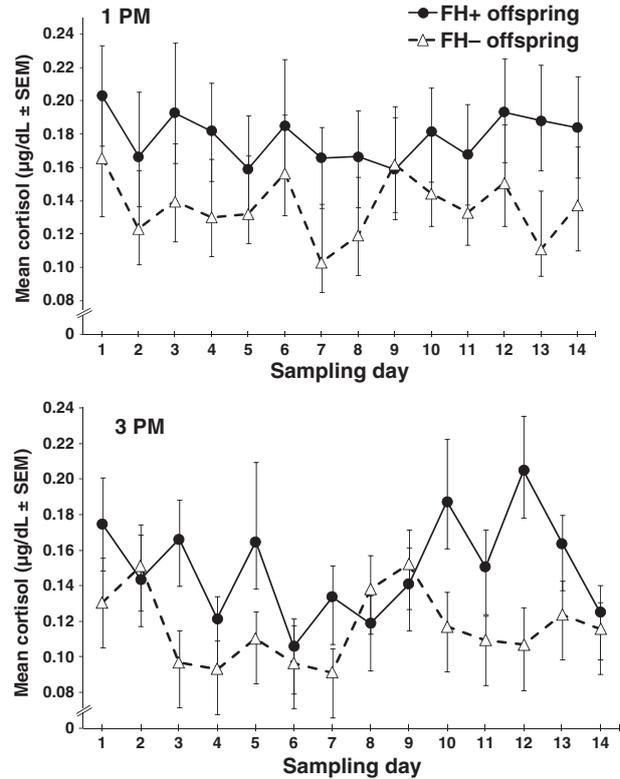


Fig. 1. Mean (\pm SEM) salivary cortisol levels at 1300h (top) and 1500h (bottom) over 14 consecutive days of sampling in the offspring of parents with bipolar disorder (FH+) and parents with no mental disorder (FH-). FH+ offspring had significantly higher levels of cortisol in the afternoon than FH- offspring.

cally significant in the previous analyses. None of the behavioral variables and their interactions with risk status were significantly associated with the cortisol intercept (data not shown), but risk status [$B = 0.019$, $SE = 0.011$, $t(33) = 1.76$, $p < 0.05$] remained a significant predictor of elevated cortisol levels, accounting for 3.9% of the between-subject variance. Thus, the relationship between risk status and cortisol levels could not be attributed to self-reported affective symptoms, behavioral problems, or daily hassles. Moreover, the relationship between cortisol and risk status was not moderated by these affective and behavioral measures.

The cortisol response following awakening, evening cortisol, and risk status

Because the afternoon data presented above included 14 days of sampling, and the awakening and evening measures included only two days of sampling, the latter data were analyzed separately. Among FH+ and FH- offspring, mean (\pm SD) cortisol levels ($\mu\text{g}/\text{dL}$) averaged over two days were, respectively, 0.232 ± 0.168 and 0.210 ± 0.134 at

awakening, 0.317 ± 0.220 and 0.306 ± 0.173 at 30 min post-awakening, 0.262 ± 0.130 and 0.262 ± 0.169 at 60 min post-awakening, 0.134 ± 0.096 and 0.095 ± 0.081 at 2000h, and 0.137 ± 0.116 and 0.118 ± 0.094 at bedtime. The multilevel analysis described in the section on afternoon cortisol, with the same covariates, was repeated first on awakening cortisol levels, and then on evening cortisol levels. Risk status was not predictive of overall cortisol levels or the slope over the three awakening samples.

For samples collected at 2000h and at bedtime, the unconditional model revealed that 53.1% of the variance in evening cortisol levels was related to within-subject variability, while the remaining amount was attributable to between-subject variability. Unlike the afternoon data, cortisol levels did not change significantly between the 2000h and bedtime samples [$B = 0.028$, $SE = 0.023$, $t(41) = 1.20$, NS]. Next, the effect of risk group was added to the between-subject model. Beforehand, sex, age, medications, subjective compliance (for the 2000h sample only), oral contraceptive use, smoking, food consumption, exercise, and lifetime diagnosis of major affective disorder were added to the model as covariates. Risk status was significantly associated with a general increase in cortisol at the 2000h sampling period [$B = 0.055$, $SE = 0.032$, $t(1\text{-tailed}, 31) = 1.71$, $p < 0.05$]. The addition of this variable led to a significant decrease in between-subject variability [$\Delta\chi^2(1) = 3.84$, $p < 0.05$], explaining 17.26% of the between-subject variance. No other between-subject factor was significantly related to cortisol levels.

In sum, consistent with the 14-day afternoon data, cortisol levels in the evening were higher in FH+ than FH- offspring. In contrast to cortisol levels in the afternoon, the cortisol response following awakening, sampled over two days, did not differ significantly between the FH+ and FH- offspring.

Stability over time

The stability of afternoon cortisol levels between the present study and our previous one (18) was examined in a subsample of participants. The Spearman rank correlation between mean log cortisol levels at 1500h over 14 days of sampling in the present study and at 1500h over two days of sampling in the previous study was not significant ($n = 32$, $r = 0.14$, NS), but it was in the expected direction. Interestingly, if only the first two days of sampling from the present study were included (so that both studies have the same number of sampling days), the above correlation increased

twofold and approached statistical significance ($n = 31$, $r = 0.34$, $p = 0.058$). Within-group correlations between the studies were modest and failed to reach statistical significance, particularly among the FH+ offspring (FH+, $n = 16$: $r = -0.14$, NS; FH-, $n = 16$: $r = 0.17$, NS). If only the first two days of sampling from the present study were included, the relationship between studies was modestly stronger (FH+, $n = 16$: $r = 0.15$, NS; FH-, $n = 15$: $r = 0.37$, NS). Because these analyses lacked statistical power, the relationships should be interpreted with due caution. In sum, the stability of cortisol levels over approximately two to three years in the full sample was modest, but in the predicted direction.

Discussion

Consistent with our prediction, the FH+ offspring, now in early adulthood, had higher levels of cortisol in the afternoon than the FH- offspring. The findings could not be attributed to group differences in affective diagnoses, subclinical symptoms, internalizing or externalizing behaviors, daily hassles, general functioning, age, the use of a medication, food consumption, exercise, and/or smoking, and were unrelated to participants' subjective compliance to the study protocol. Based on a two-day sampling protocol, cortisol levels were also elevated in the evening among FH+ offspring compared to controls. The results of this study are consistent with a previous assessment of cortisol levels in this longitudinal sample, when the participants were approximately 17 years of age on average (18). In this study, group differences in cortisol levels emerged following awakening and persisted throughout the day, with the largest group differences occurring in the afternoon. The present study is noteworthy by demonstrating persistently elevated levels of cortisol among high-risk participants over a two-week period. As observed in other 4-day (13, 35) and 10-day (11) sampling protocols, repeated sampling over long periods of time highlights stable individual differences while minimizing state-related daily fluctuations and other confounds that obscure the detection of stable group differences (36).

Some of the results of the present study were inconsistent with the previous one (18). Group differences in the cortisol response following awakening were not observed in the present study. Because morning samples were collected on only two days and the present sample size is smaller than the previous one, the analyses of cortisol following awakening may have lacked statistical power to detect a significant group difference. In

addition, sex differences in daytime cortisol levels were not observed. Elevated cortisol levels in females relative to males have been reported in adolescents (22, 37) and in some samples of children (38), but not in young adults (39). Thus, it is likely that the sex difference has dissipated over time, as the majority of participants in the present study were in young adulthood. Alternatively, the present study may have lacked statistical power to detect a significant gender difference.

The present findings are consistent with the speculation that subtle abnormalities of the HPA system may predate the development of an affective disorder. Similar conclusions have been put forth in studies of high-risk adolescents and the offspring of parents with major depression (11–13, 40), suggesting that these changes in cortisol levels are not specific to families with BD. Daytime cortisol levels and the cortisol response from awakening did not differ between 28 FH+ offspring and 33 FH– offspring (16). Because the full sample of high-risk offspring had already been treated for an affective disorder (major depression or depressive disorder not otherwise specified), this study is not comparable to the present one, with few treated cases, or other studies of nonaffected offspring (12). One speculation is that the HPA axis in affected high-risk offspring may be normalized following pharmacological treatment (41). With some exceptions (40), studies reporting abnormalities in HPA functioning among high-risk adolescents and young adults have typically examined cortisol levels sampled in the natural environment (11–13). In contrast, studies of HPA reactivity in response to psychosocial stress and the administration of CRH have not revealed consistent group differences among the offspring of parents with BD or major depression (17, 18). However, increased cortisol reactivity to the administration of CRH was observed among high-risk offspring whose mother also had an avoidant personality [i.e., introverted, neurotic, socially impaired (17)]. Thus, there may be important subgroup differences among children of parents having an affective disorder.

The significance of elevated cortisol levels in the FH+ offspring is unknown, but it has recently been reported that cortisol levels at age 13 were predictive of the development of depressive symptoms at age 16 among the offspring of parents with major depression (14). Among a small sample of FH+ offspring and controls taking part in the present longitudinal study ($n = 59$), preliminary findings indicated that 65% of participants diagnosed with a major affective disorder in young adulthood had high cortisol levels (top third of the

distribution) at the mean age of 16 or 18 years (42). High cortisol levels significantly predicted the prospective development of a major affective disorder even after controlling for the presence of disorders at the first assessment and risk status. Other studies of different high-risk populations have reported a similar relationship between elevated cortisol levels and the prospective development of major depression (13, 35) and mental health symptoms (43). In contrast, an elevated cortisol response to the administration of dexamethasone and CRH did not predict the development of an affective disorder in young adults with a first-degree relative having major depression (44). It is important to note that the small group difference in cortisol levels observed in the present study, although replicable, may have few physiological consequences. It is not known whether elevated cortisol levels represent a marker of vulnerability or a causal mechanism in the development of an affective disorder. Moreover, the within-subject stability of cortisol levels over approximately two years was modest at best. Unfortunately, the small sample of offspring with longitudinal cortisol data in this study precluded any definitive conclusions. In sum, despite the paucity of research, there is some evidence of meaningful premorbid HPA abnormalities among populations at risk for the development of an affective disorder, particularly when samples are collected in the natural environment. Still, there is a need for more definitive studies on the significance and clinical consequences of chronically elevated HPA activity.

The mechanisms by which cortisol levels are increased in young adults at high risk for major affective disorders are not known. Dysfunctions of the HPA axis may be genetically transmitted, particularly with respect to the cortisol increase after awakening (45) and/or glucocorticoid receptor function (46). Heritability estimates for afternoon cortisol levels in normal populations, however, are low (38, 47). Exposure to maternal risk factors and stressful experiences occurring prenatally (48) and during childhood (9, 11, 49) have been linked to increased HPA output in later childhood and young adulthood. In the present longitudinal cohort, parenting practices that lack consistency and organization during childhood predicted cortisol reactivity following awakening and in response to psychosocial stress during adolescence, approximately nine years later (50). Cortisol levels may also be influenced by the chronic and/or episodic stress associated with having a parent with an affective disorder. Young women at risk for depression had higher cortisol

levels if they experienced both high levels of episodic stress and poor interpersonal chronic stress; problems in either domain alone were unrelated to daytime cortisol levels (51). Indeed, FH+ offspring report more chronic interpersonal stress, particularly with family members, and a higher frequency of moderate or severe negative life events than the FH- offspring (52). Although the present study did not find a relationship between self-reported daily hassles and cortisol levels, future research should focus on objective ratings of both chronic and episodic stress. Thus, exposure to stress, either during childhood or concurrently, represents one plausible determinant of high cortisol levels in this sample.

A number of study limitations warrant consideration. First, the present findings should be interpreted with due caution, since the small sample size increases the possibility of type 1 error. However, the extensive sampling protocol (28 samples per subject) increases the stability of cortisol levels and minimizes other potential sources of error. Moreover, the results replicate a previous study in this longitudinal sample (18), and in both studies we ruled out a number of potential confounds, including oral contraceptive use, smoking, food consumption, and exercise. Second, it is difficult to attribute the observed elevations of cortisol levels in FH+ offspring as an indication of stability over time among the same individuals, or a replication of the finding among a different sample of high-risk offspring from the larger longitudinal study. This is because the sample included participants who took part in an earlier study ($n = 32$) and some who did not. Because the data on stability over time were modest, it is likely that the latter interpretation is most accurate. Third, given the high demands of this study and the low rates of psychopathology among offspring, it is possible that the present sample is not representative of the larger longitudinal sample or of families with a parent having BD in general. However, the direction of this bias would be expected to reduce the probability of finding group differences, which may explain the absence of group differences in cortisol at awakening. Fourth, compliance to the saliva collection protocol in the natural environment was subjectively but not objectively validated. However, late sampling, which was more common among FH+ than FH- participants, would confound the data in the direction of lower, not higher, cortisol levels. Another limitation of the study is the lack of alternate measures of HPA activity and of other potential biogenic influences on cortisol levels. Changes in cortisol may also indicate

changes in the levels of corticosteroid-binding globulin and/or albumin in blood (39).

In sum, the results of this 14-day naturalistic sampling protocol suggest that FH+ offspring, relative to controls, are exposed to persistently elevated levels of glucocorticoids in the afternoon and evening. Moreover, the presence of elevated cortisol levels in FH+ offspring, to some extent, persists from late adolescence to young adulthood. Although the causes of these peripheral changes are not known, elevated levels of cortisol may represent an early and subtle phenotypic marker associated with an increased vulnerability for the development of an affective disorder.

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