Similarity in Saliva Cortisol Measures in Monozygotic Twins and the Influence of Past Major Depression

Elizabeth A. Young, Steven H. Aggen, Carol A. Prescott, and Kenneth S. Kendler

Background: Some studies suggest that cortisol may be under genetic control. The aims of our study were to investigate the familial resemblance in morning and evening cortisol secretion as assessed by saliva cortisol and to assess the influence of history of major depression.

Methods: Women for this investigation were selected from an ongoing study in female–female twin pairs ascertained from the Virginia Twin Registry. Telephone screening assured that current inclusion/exclusion criteria were met. Subjects were asked to collect AM samples within 45 min after awakening, and evening samples immediately before bedtime for 14 days.

Results: There was a high degree of correlation across weeks in both the AM and PM cortisol values, indicating significant stability across individuals. There was significant correlation between AM and PM cortisol in monozygotic twins. In twins with a history of major depression (n = 30), compared with the twins without past major depression (n = 28), there was a trend towards higher cortisol (p = .056).

Conclusions: These results suggest that around 40–45% of the total variance in salivary cortisol is shared by monozygotic twins. Although the increase in baseline cortisol in twins with a history of major depression is only significant at the trend level, the effect size is comparable to an “in episode” depressed population. Biol Psychiatry 2000;48:70–74 © 2000 Society of Biological Psychiatry

Key Words: Depression, cortisol, twins, HPA, psychosocial stress

Introduction

Cortisol is the prototypical stress-responsive hormone. Despite the tendency to view cortisol secretion as related to stress, under normal circumstances cortisol secretion demonstrates a strong circadian rhythm controlled by the suprachiasmatic nucleus and linked to the wake/sleep cycle (Krieger 1979). Earlier studies demonstrating genetic control over some aspects of cortisol secretion have examined urinary, saliva, and blood steroid secretion using predominantly male twin pairs (Kirschbaum et al 1992; Linkowski et al 1993; Maxwell et al 1969; Meikle et al 1988). The aim of our study was to investigate the degree of genetic control over morning (peak) and evening (trough) cortisol secretion as assessed by saliva cortisol in women.

Increased baseline cortisol secretion in patients with major depression has been found in numerous studies (Carroll et al 1976a; Halbreich et al 1985; Pfohl et al 1985; Rubin et al 1987; Sachar et al 1973); however, it is also clear that not all patients demonstrate increased cortisol secretion. Other abnormalities of hypothalamic–pituitary–adrenal (HPA) axis regulation, including nonsuppression of cortisol to a 1-mg dexamethasone challenge, have also been reported, and again are only seen in a subgroup of patients (Carroll et al 1976b, 1981; Coppen et al 1983; Stokes et al 1984). Previous studies have suggested that dexamethasone nonsuppression is a state-dependent finding, and that it resolves when the subject is treated for depression (Grunhaus et al 1987; Ribeiro et al 1993); however, these studies have been carried out on patients while they were on antidepressant medication, and recent studies have demonstrated that antidepressants directly regulate the HPA axis by increasing glucocorticoid receptors (Holsboer and Barden 1996). This reopens the question of the state versus trait nature of the HPA axis abnormalities. To date, studies have not addressed HPA axis dysregulation in euthymic depressed patients no longer on medication. Consequently, our studies also addressed the influence of a history of major depression on saliva cortisol.

Methods and Materials

Women for this investigation were selected from an ongoing study in female–female twin pairs ascertained from the Virginia Twin Registry. All twins had been previously interviewed one or more times face to face and/or by phone by trained mental health professionals using a structured psychiatric interview that con-
tained a module, based on the Structured Clinical Interview for DSM-III-R (Spitzer and Williams 1985), for the assessment of lifetime major depression. All interviews were conducted blind to clinical information about the cotwin.

As outlined previously (Kendler et al 1992), zygosity in the larger sample was initially determined by a blind review by two experienced twin researchers that utilized standard questions (Eaves et al 1989) and photographs. Blood samples were obtained from both members of 119 pairs of uncertain zygosity and analyzed using eight restriction fragment length polymorphism markers (Spence et al 1988). More recently, we performed polymerase chain reaction (PCR) zygosity tests on an additional 269 twin pairs, oversampling those where our prior zygosity assignment was questionable. On the basis of these tests (where the mean number of markers tested per pair was 17.5 [SD = 8.4]), zygosity was changed in 12 pairs (4.5% of those tested). Of the original 30 pairs of monozygotic twins included in this study, PCR zygosities were available on 19. One of them turned out to be dizygotic and was dropped from the analyses. Monozygosity was confirmed on the remaining 18 pairs.

Female twin pairs were eligible for participation if they were monozygotic and had been cooperative when last contacted for interview, and had never met criteria for substance or alcohol abuse. Twin pairs were contacted sequentially and screened for the following criteria: all twin pairs were concordant for menopausal status. No subjects were taking oral contraceptives. None were on any current psychotropic medication, nor any steroid-containing medication. All were medically healthy. Other exclusions for participation in the study include shift work and recent jet travel, or recent illness/infections. Recruitment continued until 30 pairs were screened and agreed to participate in the saliva cortisol collection. Subjects were instructed to collect the AM samples within 45 min after awakening, before eating or brushing teeth. The evening samples were collected immediately before bedtime. In addition, at the end of each day each recorded daily hassles, mood states, and consumption of medications, alcohol, and nicotine. The hassles were rated on a scale of 1–3 and included criticism at work/school; a lot of pressure at work/school; problem or tension with coworker, spouse, friend, neighbor, or relative; illness or injury in self, spouse, or family member; overloaded with household responsibilities or other family obligations; and transportation problems. Saliva was collected with Salivette tubes (Sarstedt, Newton, NC) and stored in the refrigerator until completion of the study. All samples were mailed by the subject directly to the assay laboratory (Michigan). Previous studies in our laboratory and elsewhere have validated the stability of saliva cortisol for as long as 1 week at 37°C. Samples were collected in late fall/early winter to avoid exposure to extremely high temperatures in shipment. Each subject collected daily AM and PM samples for a 2-week period. This long period of sampling was chosen to minimize day-to-day variation that occurs due to transient environmental stressors. Saliva cortisol was assayed with DPC (Los Angeles) Coat-a-count Cortisol assay kits, using 200 μL of sample.

**Results**

Of the 30 monozygotic twin pairs recruited, one pair was reclassified as dizygotic, yielding a final sample of 58 subjects (29 pairs). Of the possible 812 morning samples, 58 samples were missing. Of the possible 812 evening samples, 54 were missing, demonstrating high compliance with the protocol. Cortisol values for 10 of the 1512 samples were extremely high, beyond the physiologic range (outliers), and were deleted from the data set before analysis.

The mean saliva cortisol across all subjects was 4.17 ± 0.2 ng/mL in the AM and 1.73 ± 0.1 ng/mL in the PM. Table 1 demonstrates the within-subject correlation between the averaged values from weeks 1 and 2. As can be seen, there is a high correlation across weeks in both the AM and PM mean cortisol values, as well as their standard deviations, indicating a significant stability within individuals. Table 2 shows the cross-twin correlation across weeks for the mean of 2 weeks and for the mean of week 1 only. As can be seen, there is a significant twin-pair correlation for AM and PM cortisol in monozygotic twins. These results suggest that around 40 – 45% of the total and about 50% of the reliable variance in salivary cortisol is shared by monozygotic twins. Because our sample included only monozygotic twins, it is not possible to determine the degree to which the twin resemblance is due to genetic versus shared familial–environmental factors.

<table>
<thead>
<tr>
<th>Table 1. Correlation across Weeks within Individuals</th>
<th>Week 1 cortisol (mean AM)</th>
<th>Week 1 cortisol (mean PM)</th>
<th>Week 1 (standard deviation AM)</th>
<th>Week 1 (standard deviation PM)</th>
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<tr>
<td>Week 2 Mean cortisol AM</td>
<td>.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.55&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Week 2 Mean cortisol PM</td>
<td>.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.67&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Week 2 Standard deviation cortisol AM</td>
<td>.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.62&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Week 2 Standard deviation cortisol PM</td>
<td>.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.72&lt;sup&gt;d&lt;/sup&gt;</td>
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<sup>a</sup><sup>p</sup> < .0001.<br><sup>b</sup><sup>p</sup> < .005.<br><sup>c</sup><sup>p</sup> < .005.<br><sup>d</sup><sup>p</sup> < .01.<br>

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<tr>
<th>Table 2. Correlation across Twins of Mean Cortisol</th>
<th>Mean AM cortisol twin 1</th>
<th>Mean PM cortisol twin 1</th>
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<tr>
<td>Both weeks Mean AM cortisol twin 2</td>
<td>.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.47&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mean PM cortisol twin 2</td>
<td>.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.42&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Week 1 Mean AM cortisol twin 2</td>
<td>.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.52&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mean PM cortisol twin 2</td>
<td>.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup><sup>p</sup> < .004.<br><sup>b</sup><sup>p</sup> < .0067.
mean daily mood ratings showed little correlation with mean AM cortisol (.13, \( n = 58, p = .33 \)) or mean PM cortisol (.002, \( n = 58, p = .99 \)).

Finally, the twins with a lifetime history of major depression (\( n = 30 \)) were compared to the twins without past major depression (\( n = 28 \)). The data are shown in Figure 1. There was the expected significant difference between AM and PM cortisol values [ANOVA, \( F(1) = 99.0, p = .0001 \)]. There was a trend towards higher cortisol in individuals with a history of major depression [ANOVA, \( F(1) = 3.72, p = .056 \)]. Examining the distribution of mean saliva cortisol (mean of AM and PM), blind to the diagnosis of the subject, we realized that there was one twin pair with very low saliva cortisol values, and these values were 50% lower than the next lowest individuals (outliers). Both twins had a lifetime history of major depression. If these two individuals are deleted from the analysis, the difference in saliva cortisol between twins with a lifetime history of major depression and twins without a history is significant [\( F(1) = 8.0, p = .005 \)], and the magnitude of increase in cortisol in the remaining individuals with a lifetime history of major depression is now 23%.

Because a number of twin pairs were discordant for major depression (\( n = 12 \)), we examined the mean cortisol in the unaffected twins compared with twin pairs in which neither twin had a history of major depression (Table 3). As can be seen, the saliva cortisol in the AM in well twins from pairs discordant for major depression appears to be intermediate (.41) compared with those without a cotwin with a lifetime history of major depression (.37) or twins with a diagnosis of MDD (.45), although none of these differences were significant.

**Discussion**

Saliva cortisol is a simple, noninvasive means of examining cortisol in response to everyday life situations. Given the stability of the samples at room temperature, the collection of saliva cortisol is easily undertaken in an epidemiologic population. The cooperation rate was extremely high (7% missing samples). The group means demonstrate the expected circadian rhythm, suggesting little problems in time of sample collection. Since we collected saliva cortisol for 2 weeks, we were able to compare weeks 1 and 2 within individuals to assess the stability of cortisol across weeks, as well as to compare the correlation within twins to across twins. The stability shown in Table 1 is quite high. The data in Table 2 for within-pair correlation demonstrate a correlation coefficient of half that seen within twins (AM = .78 within individuals, .47 across twins; PM = .89 within individuals, .418 across twins). Since we only studied monozygotic twins, it is possible that the high correlation seen between all twin resemblance resulted from genetic factors, our findings would suggest a heritability for mean cortisol values, assessed by salivary cortisol, in the range of 40–50%.

The relationship between daily hassles and AM and PM cortisol was examined in several ways. The correlations of mean daily hassles with mean AM and PM cortisol values were −.136 (\( n = 58, p = .31 \)) for the AM and −.178 (\( n = 58, p = .18 \)) for the PM, suggesting no relationship between average hassles and mean cortisol. If all cortisol values and all hassles scores are entered into the correlation there is similarly no relationship between hassles and cortisol reading in the AM or PM \( (r = -.069, n = 754, p = .06 \) in the AM; \( r = -.059, n = 758, p = .10 \) in the PM). The last analyses compared within individuals the mean cortisol on days with hassles to mean cortisol on days without hassles. Fourteen subjects reported no or only mild hassles on any day. The remainder of subjects (44) reported hassles labeled from moderate (2) to very stressful (3). Only days with hassles rated moderate to severe and occurring after noon were examined. The cortisol means of these days were compared to the means of days without any hassles, using an analysis of variance (ANOVA) with diagnoses, time, and hassles as three factors. There was no significant effect of hassles \( (F = 0.43, p = .51) \) on AM and PM cortisol, nor was there a significant interaction \( (p = .62) \). If the definition of hassles is restricted to severe events only occurring after noon, the sample size decreases to 36, but there was still no effect of hassles on saliva cortisol \( (p = .7) \). Although no twins currently met criteria for depression, ratings of mood were collected on a daily basis during the study. The

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**Figure 1.** Saliva cortisol in subjects without a history of major depressive disorder (*) versus those with a past history of major depressive disorder (○).
saliva cortisol. There was no association between current mood measures and bedtime cortisol measures was too long. Similarly, there because the time lag between the stressful event and the influential influenced the evening cortisol values. This may be on cortisol in several ways. In no case could we find evidence of a genetic component for mean cortisol in 10 monozygotic and 10 dizygotic twins, since the correlations for both dizygotic and monozygotic twin pairs were both extremely high. The study of Kirschbaum et al (1992) examined afternoon saliva cortisol and the plasma cortisol response to three challenges: corticotropin-releasing factor (CRF), bicycle ergonometry, and a social stressor. They found evidence of genetic control over baseline 4:00 PM cortisol but failed to find a genetic component to the response to the social stressor, since the correlations between both monozygotic and dizygotic twins were high. The possibility that shared early experience may influence HPA axis responsiveness is in agreement with basic science studies on persistent effects of maternal deprivation or separation on the HPA axis (Ladd et al 1996; Sutanto et al 1996). Such hypotheses are fruitful areas for further exploration.

Having demonstrated twin resemblances in basal AM and PM cortisol, we examined the effects of daily hassles on cortisol in several ways. In no case could we find evidence that events coded as moderate to severely stressful influenced the evening cortisol values. This may be because the time lag between the stressful event and the bedtime cortisol measures was too long. Similarly, there was no association between current mood measures and saliva cortisol.

The finding of high correlation within twin pairs allows us to examine the question of the relationship between cortisol and history of depression. Although baseline cortisol is increased in a subgroup of “in episode” depressed patients, unanswered questions include whether this abnormality is a state marker or a trait marker, and whether the increase is a marker of vulnerability or a consequence of illness. Older studies addressed the question of trait markers by examining patients following treatment with tricyclic antidepressants; however, the realization that antidepressants themselves directly regulate the HPA axis, including increasing concentrations of glucocorticoid receptors in feedback brain circuitry (Holsboer and Barden 1996), suggests that previous studies examining the trait nature of this abnormality need to be reconsidered. The data presented here demonstrate a 15% increase in free cortisol in the AM and a 25% increase in the PM in well twins with a history of major depression. This increase is similar to that observed in a group of 22 drug-free depressed women undergoing sampling for corticotropin and cortisol every 10 min (E.A. Young, unpublished data, 1999). Although the difference is only significant at the trend level, the effect size is comparable to an “in episode” depressed population, strongly suggesting an effect of history of major depression on the HPA axis as reflected by saliva cortisol. Furthermore, there is substantial heterogeneity in the depressed population, since one twin pair demonstrated extremely low cortisol. We have previously reported on a small subgroup of currently depressed patients who had low cortisol values before a CRF challenge. In addition, low mean baseline cortisol has been found in patients with chronic fatigue, patients with fibromyalgia, and posttraumatic stress disorder subjects even when currently depressed (Crofford et al 1994; Demitrack et al 1991; Tsigos and Chrousos 1994; Yehuda et al 1995, 1996). Thus, the probability of more than one type of HPA axis dysregulation in depression is clear.

Given the evidence of an effect of history of major depression on saliva cortisol, the next most important question is whether this is a consequence of depression or a reflection of underlying vulnerability. Since it is difficult to assess high-risk individuals and then observe them longitudinally to ascertain who gets depressed, the use of twin pairs discordant for depression provides a unique opportunity to assess the correlation of “genes for major depression” with saliva cortisol. We found no significant differences between well individuals in twin pairs concordant or discordant for major depression, although the sample size was extremely small. A more comprehensive study with larger sample sizes is needed to address this question.

In conclusion, our data suggest that AM and PM saliva cortisol measures show strong familial resemblance. In addition, individuals with a history of major depression who are currently well show evidence of increased saliva cortisol. Since we did not assess baseline HPA axis function during a depressive episode in these individuals, we do not know if the observed increases in subjects with

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<th>AM</th>
<th>PM</th>
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<tr>
<td>MDD All</td>
<td>0.45 ± 0.03</td>
<td>0.19 ± 0.12</td>
</tr>
<tr>
<td>Non-MDD All</td>
<td>0.39 ± 0.02</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>MDD subjects</td>
<td>0.44 ± 0.04</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Discordant (MDD - cotwin)</td>
<td>0.46 ± 0.05</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Non-MDD subjects</td>
<td>0.37 ± 0.03</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>Discordant (MDD + cotwin)</td>
<td>0.41 ± 0.04</td>
<td>0.16 ± 0.05</td>
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a history of MDD represent continued HPA axis activation in the same individuals or if the liability to depression predisposes to activation of the HPA axis that may occur on a sporadic basis both in and out of episode.

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**References**


