

Parental Posttraumatic Stress Disorder as a Vulnerability Factor for Low Cortisol Trait in Offspring of Holocaust Survivors

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Context: Lower cortisol levels in posttraumatic stress disorder (PTSD) may reflect a preexisting vulnerability associated with developing the disorder after trauma exposure. Because offspring of trauma survivors with PTSD have a greater prevalence of PTSD after their own life events than offspring of trauma survivors without PTSD and offspring of nonexposed persons, examination of patterns of basal cortisol secretion in such offspring provides an opportunity to test this hypothesis.

Objective: To characterize the patterns of basal cortisol secretion in offspring of Holocaust survivors with and without parental PTSD and children of nonexposed parents.

Design: Cortisol secretion was measured every 30 minutes for 24 hours. The raw hormonal data were subjected to a chronobiological analysis by applying single-oscillator and multioscillator cosinor analyses, a nonlinear least squares curve-fitting program, to determine circadian and ultradian regulatory dynamics.

Setting: The study was conducted under controlled conditions at the General Clinical Research Center at the Mount Sinai School of Medicine.

Participants: Twenty-three Holocaust offspring with parental PTSD and 10 without parental PTSD were compared with 16 children of nonexposed parents. No participant had PTSD.

Main Outcome Measures: Mean cortisol levels during the 24-hour cycle and other chronobiological parameters (amplitude, acrophase, circadian quotient, and goodness-of-fit coefficient) derived from single-oscillator and multioscillator models.

Results: Offspring with parental PTSD displayed lower mean cortisol levels, reflected by the circadian mesor and reduced cortisol amplitude, compared with offspring without parental PTSD and children of nonexposed parents. This effect seemed to be specifically related to the presence of maternal PTSD.

Conclusions: Low cortisol levels and other chronobiological alterations in offspring are associated with the risk factor of maternal PTSD, raising the possibility that these alterations are acquired via glucocorticoid programming either from in utero exposures or in response to maternal behaviors early in life.

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PHENOMENOLOGICAL AND BIOLOGICAL differences between trauma survivors with and without posttraumatic stress disorder (PTSD) are generally considered consequences of the traumatic event that precipitated PTSD or correlates of symptom expression. However, any variable that distinguishes trauma-exposed persons with PTSD from those without PTSD might be a pretraumatic risk factor for the disorder. Once identified, such risk factors may prove to be useful as predictors of who will develop PTSD after exposure to trauma, or they may even identify potential new targets for prophylaxis and treatment.

Examination of biological correlates of pretraumatic risk factors is warranted be-

cause trauma exposure alone does not fully explain why PTSD develops.¹ Thus, a broader range of factors, including genetic or epigenetic modifications, might explain why reinstatement of physiologic homeostasis does not occur after trauma in individuals who develop PTSD.

Although there are no established biological risk factors for PTSD, emerging evidence suggests that cortisol-related alterations may reflect increased vulnerability to development of the disorder after trauma exposure. Reduced cortisol levels in chronic PTSD have been reported in many, but not all, studies.² However, longitudinal investigations³⁻⁵ have demonstrated an association between lower cortisol levels in the acute aftermath of trauma and the subsequent development of PTSD or the

risk factor of previous trauma exposure. Because cortisol release in response to stress inhibits other stress-induced biological responses, it has been suggested that reduced cortisol levels during trauma exposure permit an extended psychophysical distress response that facilitates the development of PTSD.^{6,7}

Parental PTSD seems to be a relevant risk factor for PTSD, as evidenced by a greater prevalence of PTSD, but not trauma exposure, in adult offspring of Holocaust survivors with PTSD compared with children of Holocaust-exposed parents without PTSD.⁸⁻¹² Our group previously reported that offspring of Holocaust survivors with PTSD showed significantly lower mean 24-hour urinary cortisol excretion¹³⁻¹⁵ and enhanced cortisol suppression in response to low dexamethasone administration¹⁶ than offspring of Holocaust survivors without PTSD. Urinary cortisol excretion and plasma cortisol suppression after dexamethasone treatment were negatively correlated with the severity of parental PTSD symptoms, even after controlling for PTSD and other symptoms in offspring.^{15,16} Lower salivary cortisol levels were also observed in infants of mothers who developed PTSD vs those who did not develop PTSD after exposure to the World Trade Center collapse on September 11, 2001 (9/11), while pregnant, particularly if exposed during the third trimester.¹⁷ Here too there was a negative correlation between the severity of maternal PTSD and cortisol levels in the infant offspring. That this effect could be observed so early in the life of an offspring supports the proposition that cortisol levels are associated with the risk factor of parental PTSD.

The aim of the present study is to further characterize the pattern of basal cortisol secretion in offspring of Holocaust survivors with and without parental PTSD compared with Jewish adult children of non-trauma-exposed parents. Because cortisol release is strongly affected by circadian and ultradian rhythms,¹⁸⁻²⁰ we conducted a chronobiological analysis to elucidate the intrinsic regulatory dynamics controlling cortisol levels under baseline conditions. We previously used this approach to demonstrate that combat veterans with PTSD had significantly different patterns of cortisol secretion and regulation across the diurnal cycle compared with nonpsychiatric comparison subjects and patients with depression, reflecting a more sensitized cortisol system (ie, in contrast to the more dysregulated pattern observed in depressed patients consistent with desensitization of the hypothalamic-pituitary-adrenal [HPA] axis).²⁰ We hypothesized that alterations in plasma cortisol levels and chronobiological parameters would be present only in offspring with parental PTSD. In secondary analyses, we examined the effects of having 1 vs 2 parents with PTSD (ie, "dose" of exposure to parental symptoms) and explored the contribution of maternal vs paternal PTSD to these effects.

METHODS

PARTICIPANTS

Twenty-three men and 26 women participated in the study. Recruitment was through advertisements requesting Jewish volunteers for research examining transgenerational effects of the

Holocaust in the New York area. The procedures were approved by the institutional review boards of Mount Sinai School of Medicine and James J. Peters Veterans Affairs Medical Center. Written informed consent was obtained from all the participants.

Offspring were born after World War II or (in 4 cases) after their parents had escaped to safety during the war and were raised through adolescence by biological parents who had been interned in a Nazi concentration camp during World War II or faced comparably severe threats in hiding. These participants were further subdivided based on whether at least 1 parent met the diagnostic criteria for lifetime PTSD according to the Parental PTSD Questionnaire (PPQ), completed by the offspring.²¹ This scale was recently validated against 58 clinical interviews of the parents using the Clinician-Administered PTSD Scale, Lifetime (CAPS-L) and was found to have good convergent validity for PTSD diagnosis.²¹ The PTSD diagnosis was determined on the basis of a positive endorsement of at least 6 symptoms distributed in the 3 required categories according to DSM-IV criteria.²² For 2 offspring who did not complete the PPQ, assignment to the parental PTSD group was made on the basis of clinical interviews of parents using the CAPS-L.

Comparison subjects were Jewish, of comparable age as offspring (born from 1937 to 1974), and from the same communities but with parents who were not exposed to the Holocaust (or other major traumatic events, such as war, rape, or torture). All the participants were born to parents of European or American descent, with 73% of offspring and 87% of comparison subjects born in the United States or Canada.

Participants were not included if they had a history or evidence of psychotic illness or bipolar disorder; current alcohol or substance dependence; major medical, endocrinologic, or neurologic illness likely to interfere with HPA axis function; or laboratory data indicating acute or chronic disease. Current or lifetime mood or anxiety disorder was not an exclusion criterion. Participants were not withdrawn from medication therapy, and 6 participants (4 offspring with parental PTSD, 1 offspring without parental PTSD, and 1 control) were stabilized by receiving venlafaxine hydrochloride ($n=2$), fluoxetine hydrochloride ($n=2$), or clonazepam ($n=1$) or by self-medicating with ginkgo biloba ($n=1$). No participants were taking oral or topical glucocorticoid preparations; none were active smokers.

CLINICAL ASSESSMENTS

Axis I diagnoses were made by trained psychologist or psychiatrist raters using the Structured Clinical Interview for DSM-IV²³ and were confirmed by consensus conference. Information about lifetime traumatic events was obtained using the Trauma History Questionnaire.²⁴ The symptom severity of PTSD was determined using the CAPS-L.²⁵ Participants also completed the Childhood Trauma Questionnaire (CTQ)²⁶ for the assessment of traumatic experiences, such as neglect and abuse, before age 15 years.

PROCEDURE

Participants were admitted to the Mount Sinai General Clinical Research Center on the evening before the study. The next morning a catheter was inserted at 5:30 AM, and after a stabilization period of approximately 60 minutes, blood samples were obtained every 30 minutes for 24 hours. Participants were kept supine for the duration of the study. To avoid large postprandial increases in cortisol levels in response to meals,

each participant received standardized low-calorie meals at 9 AM, noon, and 6 PM. Participants were kept awake until 11 PM, when the lights were switched off until 6:30 AM the following morning. Participants were awakened at 6:30 AM and were provided with breakfast after the last blood sample collection. The sleep/wake status of each participant was recorded every 30 minutes.

BIOLOGICAL MEASURES

Plasma cortisol levels were determined by means of radioimmunoassay using a commercially available kit (DiaSorin Inc, Stillwater, Minnesota). The intra-assay and interassay coefficients of variation for this method in Dr Yehuda's laboratory are 4.0% and 6.8%, respectively.

DATA ANALYSIS

The groups were compared using χ^2 tests for categorical descriptors, analyses of variance or covariance for continuous variables, and repeated-measures analysis of covariance with Huynh-Feldt adjustment for degrees of freedom for the analysis of the 48 cortisol values throughout the day. Age, sex, body mass index (BMI) (calculated as the weight in kilograms divided by height in meters squared), medication status, presence of mood or anxiety disorder, and lifetime PTSD symptom severity were tested individually for association with the biological outcome measures. Age and sex were not included as covariates because neither was associated with cortisol level (age: $r = -0.12$, $n = 49$; $P = .40$; sex [mean \pm SD]: men, 8.05 ± 0.39 $\mu\text{g/dL}$ [to convert to nanomoles per liter, multiply by 27.588]; women, 8.17 ± 0.36 $\mu\text{g/dL}$; $F_{1,46} = 0.05$; $P = .82$). The BMI was significantly associated with mean cortisol level ($r = -0.35$, $n = 49$; $P = .01$) and was used as a covariate for all the analyses. There were no differences in mean \pm SD cortisol levels between those taking (8.10 ± 2.2 $\mu\text{g/dL}$) and not taking (8.11 ± 1.9 $\mu\text{g/dL}$) psychotropic medications ($t_{47} = 0.001$; $P = .98$). In fact, the main findings described herein remained unchanged when participants taking psychotropic medications were removed from the analyses. The presence or absence of depression or anxiety diagnoses and lifetime PTSD symptom severity as reflected by the CAPS-L total score were used as covariates to eliminate the possible association of offspring symptom expression with cortisol levels from analyses of the effects of parental PTSD. This approach was supported by the significance of the associations in the offspring subgroup between mean cortisol levels and the presence of a depression or anxiety diagnosis ($r_{29} = 0.42$; $P = .02$, controlling for PTSD lifetime symptom severity and BMI) and between mean cortisol levels and PTSD symptom severity ($r_{29} = -0.39$; $P = .03$, controlling for depression or anxiety diagnosis and BMI).

To evaluate differences in chronobiological factors, raw cortisol data were modeled using standard oscillator and multioscillator cosinor models using the COSIFIT program.¹⁸ This program applies a nonlinear least squares curve-fitting routine to the data. The single-oscillator model determined the best-fitting 24-hour cosine function and provided information on the mesor (mathematically corrected mean cortisol level across the 24-hour cycle), amplitude (highest cortisol level), acrophase (time of peak), quotient (amplitude-mesor ratio signifying "signal-to-noise"), and goodness of fit (the coefficient yielding the correlation between the raw cortisol values and the curve to which the data were being fit) of this rhythm. The multioscillator model included additional cosine functions to evaluate ultradian (ie, shorter and nondiurnal) fluctuations in cortisol release and included a 12-hour (hemircadian) component that represents the nonsinusoidal

component of the circadian rhythm and an expression of cortisol rhythmicity.

RESULTS

CHARACTERISTICS OF THE SAMPLE

Sample characteristics and results of group comparisons are summarized in **Table 1**. There were no significant group differences in age, sex distribution, years of education, BMI, exposure to criterion A traumatic events, or childhood trauma as measured using the CTQ. Although statistical significance for sex was not achieved with this sample size, the proportion of men in the comparison group was nearly double that of offspring with parental PTSD and almost as large relative to offspring without parental PTSD. Similarly, there were no significant group differences in the percentage of participants with mood and anxiety disorders, although the proportion with these diagnoses among offspring with parental PTSD was more than twice that of the other 2 groups. No participant met the diagnostic criteria for current or lifetime PTSD. The CAPS-L ratings reflecting lifetime PTSD symptom severity in offspring did not differ significantly among the groups, but scores for the offspring group with parental PTSD averaged more than 50% higher than those of the other 2 groups when total scores or maternal and paternal subscales were considered. Offspring with parental PTSD showed nonsignificantly higher ratings for total CTQ scores, whereas scores for offspring without parental PTSD and comparison subjects were similar. Finally, comparison subjects reported nonsignificantly more criterion A traumatic events than either offspring group.

Offspring with parental PTSD endorsed significantly more negative consequences of being raised by Holocaust survivor parents than those without parental PTSD, but the groups were comparable with respect to positive effects. Offspring ratings of PTSD in their mothers and fathers are also given in Table 1. To determine whether there were differences in offspring ratings of maternal vs paternal PTSD, a paired t test was performed in the offspring sample as a whole. This analysis demonstrated no difference between ratings of mean \pm SD maternal PTSD (15.90 ± 14.5) and paternal PTSD (13.29 ± 11.05) ($t_{30} = 0.82$; $P = .42$). Scores for maternal and paternal PTSD were not correlated ($r = 0.04$, $n = 31$; $P = .83$). Similar results (mean \pm SD) were obtained when comparing the subsample ($n = 8$) in whom both parents were rated as having PTSD (maternal PTSD score: 24.75 ± 10.99 ; paternal PTSD score: 23.62 ± 8.34 ; $t_7 = 0.24$; $P = .24$). In this subsample, scores for maternal and paternal PTSD also were not correlated ($r = 0.10$, $n = 8$; $P = .82$).

EFFECT OF PARENTAL PTSD ON PLASMA CORTISOL LEVELS

Figure 1 depicts the raw cortisol values for the 3 groups across the 24-hour period. Two-way repeated-measures analysis of covariance revealed main effects of group ($F_{2,43} = 5.72$; $P = .006$; partial $\eta^2 = 0.21$) and time

Table 1. Characteristics of Offspring With and Without Parental PTSD and Nonexposed Comparison Subjects

	Offspring With Parental PTSD (n = 23)	Offspring Without Parental PTSD (n = 10)	Comparison Subjects (n = 16)	F or χ^2	P Value
Age, mean \pm SD, y	47.0 \pm 7.8	39.0 \pm 9.9	46.6 \pm 17.5	$F_{2,46} = 1.66$.20
Male sex, %	35	40	69	$\chi^2_2 = 4.61$.10
Education, mean \pm SD, y	17.6 \pm 1.6	17.3 \pm 3.7	17.2 \pm 2.9	$F_{2,46} = 0.17$.85
BMI, mean \pm SD	25.2 \pm 3.5	25.0 \pm 4.3	25.4 \pm 4.6	$F_{2,46} = 0.03$.97
Criterion A trauma, %	35	30	56	$\chi^2_2 = 2.41$.30
CTQ score, mean \pm SD ^a	9.6 \pm 3.0	7.5 \pm 3.2	7.9 \pm 2.4	$F_{2,35} = 2.06$.14
CAPS-L score, mean \pm SD ^b	22.7 \pm 22.0	13.8 \pm 19.7	14.9 \pm 21.0	$F_{2,46} = 0.94$.40
Mood or anxiety disorder, %	48	20	19	$\chi^2_2 = 4.54$.27
PPQ, mean \pm SD	35.5 \pm 14.9	12.0 \pm 11.3	NA	$F_{1,29} = 19.56$	<.001
Maternal score	19.9 \pm 14.6	7.6 \pm 10.6	NA	$F_{1,29} = 5.62$.03
Paternal score	17.3 \pm 10.7	4.9 \pm 6.1	NA	$F_{1,29} = 11.47$.002
Negative effects ^{c,d}	11.4 \pm 2.8	7.4 \pm 3.8	NA	$F_{1,27} = 10.40$.003
Positive effects ^{c,e}	9.7 \pm 3.0	7.9 \pm 2.5	NA	$F_{1,27} = 2.71$.09

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAPS-L, Clinician-Administered PTSD Scale, Lifetime; CTQ, Childhood Trauma Questionnaire; NA, not applicable; NS, not significant; PPQ, Parental PTSD Questionnaire; PTSD, posttraumatic stress disorder.

^aTo examine differences in CTQ scores based on maternal or paternal PTSD in offspring, a 2-way analysis of variance was performed using maternal and paternal PTSD as between-subject factors. There was no significant main effect of either maternal ($F_{1,23} = 2.88$; $P = \text{NS}$) or paternal ($F_{1,23} = 0.009$; $P = \text{NS}$) PTSD, nor was the interaction of maternal and paternal PTSD significant ($F_{1,23} = 0.62$; $P = \text{NS}$), reflecting no differences in mean \pm SD CTQ scores among offspring with (9.9 \pm 3.0) or without (7.8 \pm 2.9) maternal PTSD and with (9.1 \pm 3.2) or without (8.7 \pm 3.2) paternal PTSD. Comparison of mean \pm SD CTQ total scores for offspring with maternal PTSD only (10.6 \pm 2.5; $n = 6$) vs paternal PTSD only (8.4 \pm 2.6; $n = 4$) was not significant ($t_6 = 1.38$; $P = \text{NS}$).

^bThese scores reflect worst-episode PTSD symptom severity experienced by the offspring after exposure to trauma. No participant met the diagnostic criteria based on 1 intrusive, 3 avoidance, and 2 hyperarousal symptoms after a criterion A event.

^cThese questions were unscored in 2 individuals who did not complete this section of the test.

^dThis score reflects the sum of 3 items on the PPQ rated on a 5-point Likert scale: "I have vicariously experienced the trauma of the Holocaust through my parents," "I have psychological scars because of my parents," and "I am more likely to be affected by stress as a result of my parents' experiences."

^eThis score reflects the sum of 3 items on the PPQ: "I have unique strengths because of my parents," "I am more sensitive to violence and injustice because of my parents' experiences," and "I am more resilient to stress because of my parents."

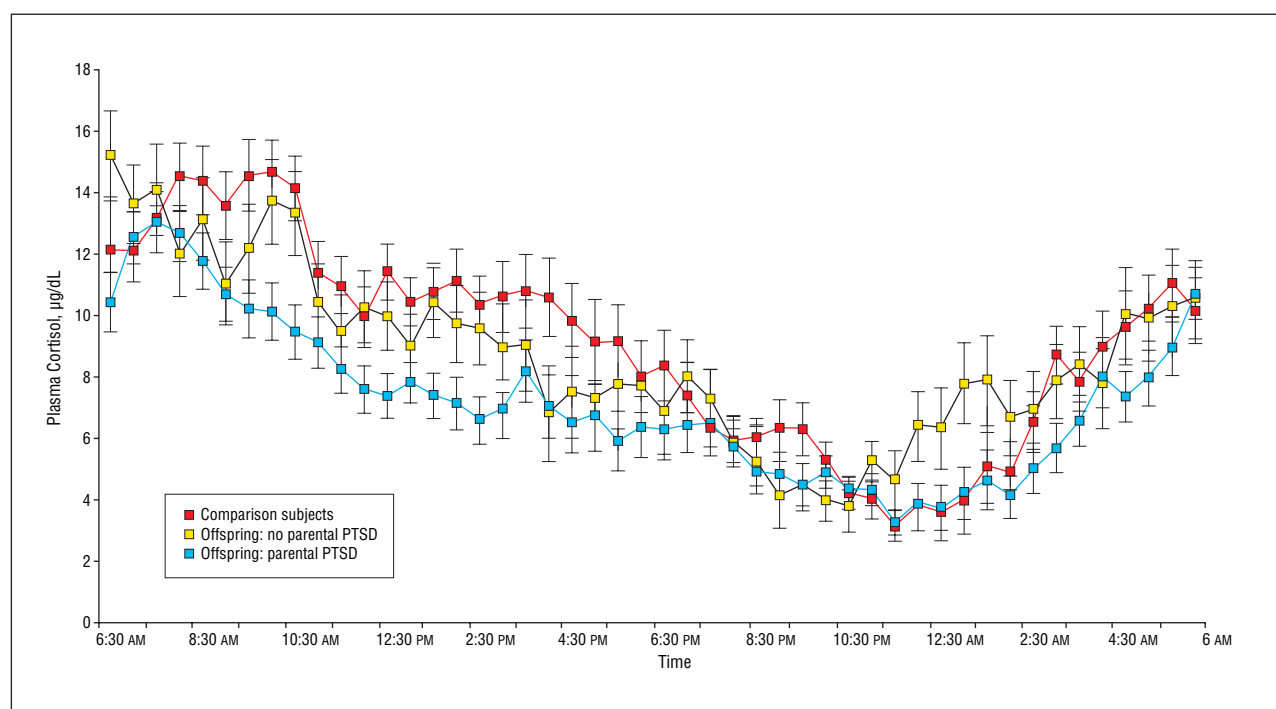


Figure 1. Mean plasma cortisol values of Holocaust offspring with and without parental posttraumatic stress disorder (PTSD) and of comparison subjects. The estimated mean \pm SE plasma cortisol levels were 8.92 \pm .41 $\mu\text{g/dL}$ for controls, 8.84 \pm 0.52 $\mu\text{g/dL}$ for offspring without parental PTSD, and 7.23 \pm 0.35 $\mu\text{g/dL}$ for offspring with parental PTSD. Error bars represent SE. To convert cortisol to nanomoles per liter, multiply by 27.588.

($F_{11.94,513.38} = 2.65$; $P = .002$; partial $\eta^2 = 0.06$) but no significant group \times time interaction ($F_{23.88,513.38} = 1.12$; $P = .25$). Both BMI ($F_{1,43} = 8.99$; $P = .004$) and a current depression

or anxiety diagnosis ($F_{1,43} = 8.49$; $P = .006$) were significant covariates, whereas lifetime total score on the CAPS-L was not ($F_{1,43} = 0.41$; $P = .52$).

Table 2. Summary of Chronobiological Parameters^a

	Offspring With Parental PTSD (n = 23)	Offspring Without Parental PTSD (n = 10)	Comparison Subjects (n = 16)	P Value					
				ANCOVA ^b			P Value		
				F _{2,43}	P Value	Partial η^2	Parental PTSD vs Comparison	Parental PTSD vs No Parental PTSD	No Parental PTSD vs Comparison
Cosinor model									
Mesor ^c	7.12 ± 0.35	8.72 ± 0.52	8.63 ± 0.41	5.00	.01	0.19	.009	.02	.89 ^d
Circadian amplitude	3.47 ± 0.32	4.19 ± 0.48	4.77 ± 0.38	3.26	.048	0.13	.02	.23	.34
Circadian acrophase	9:32:57 ± 2:49:14	9:46:36 ± 1:46:17	10:27:30 ± 1:54:53	0.41	.67	NA	NA	NA	NA
Circadian quotient ^e	0.50 ± 0.03	0.51 ± 0.05	0.55 ± 0.04	0.53	.59	NA	NA	NA	NA
Goodness of fit (r)	0.65 ± 0.03	0.72 ± 0.04	0.74 ± 0.03	2.58	.09	0.11	.035	.16	.66
Multicosinor model									
Mesor ^f	7.14 ± 0.35	8.69 ± 0.52	8.62 ± 0.42	4.70	.01	0.18	.01	.02	.92
Circadian amplitude	3.56 ± 0.32	4.31 ± 0.48	4.77 ± 0.38	2.95	.06	0.12	.02	.21	.44
Circadian acrophase	9:37:02 ± 2:50:56	9:49:13 ± 2:06:06	10:12:01 ± 1:57:10	0.07	.93	NA	NA	NA	NA
Circadian quotient ^g	0.51 ± 0.03	0.52 ± 0.05	0.50 ± 0.04	0.34	.71	NA	NA	NA	NA
Hemicircadian amplitude	2.64 ± 0.30	1.85 ± 0.45	2.96 ± 0.36	1.46	.24	NA	NA	NA	NA
Hemicircadian quotient	0.35 ± 0.03	0.22 ± 0.05	0.23 ± 0.04	3.47	.04	0.14	.03	.04	.94
Ultradian amplitude	2.26 ± 0.25	1.87 ± 0.37	2.07 ± 0.30	0.38	.69	NA	NA	NA	NA
Ultradian frequency	4.11 ± 0.44	5.31 ± 0.66	4.17 ± 0.52	1.26	.29	NA	NA	NA	NA
Ultradian quotient	0.31 ± 0.03	0.22 ± 0.04	0.23 ± 0.03	2.44	.10	0.10	.08	.07	.79
Goodness of fit (r)	0.88 ± 0.01	0.87 ± 0.02	0.88 ± 0.01	0.25	.78	NA	NA	NA	NA

Abbreviations: ANCOVA, analysis of covariance; NA, not applicable; PTSD, posttraumatic stress disorder.

^aData are given as mean ± SE unless otherwise indicated.

^bControlling for body mass index (BMI), current depression or anxiety disorder diagnoses, and Clinician-Administered PTSD Scale total lifetime score.

^cSignificant covariates were BMI ($P = .004$) and current depression or anxiety disorder diagnoses ($P = .006$).

^dAt $P \geq .10$.

^eThe significant covariate was BMI ($P = .03$).

^fSignificant covariates were BMI ($P = .005$) and current depression or anxiety disorder diagnoses ($P = .004$).

^gThe significant covariate was BMI ($P = .04$).

EFFECT OF PARENTAL PTSD ON CHRONOBIOLOGICAL PARAMETERS

Results of the chronobiological analyses are summarized in **Table 2**. Cosinor analyses revealed significant group differences in the mesor and amplitude of the circadian waveform but no significant differences in the circadian quotient or acrophase. The mesor was 18.3% lower in offspring with parental PTSD compared with those without parental PTSD and 17.5% lower than in controls. The amplitude in offspring with parental PTSD was 27.2% lower than that in controls and 17.2% lower (non-significantly) than that in offspring without parental PTSD. A trend for a group difference in the goodness-of-fit coefficient indicated a worse fit of the data to a circadian model for offspring with parental PTSD than controls. None of the parameters differentiated the offspring group without PTSD from comparison subjects.

Multioscillator cosinor analyses provided substantially better goodness of fit of the data to the model in all 3 groups compared with the single-oscillator model. The differential, or improvement, in the goodness-of-fit coefficient was greatest for the offspring group with parental PTSD ($r = 0.65$ – 0.88). Multioscillator analyses also provided information about hemicircadian and ultradian parameters. Significant group differences were seen only in the hemicircadian quotient, with a trend finding for the ultradian quotient, which in both cases reflects an increased signal-to-noise ratio in offspring with parental PTSD compared with the other 2 groups.

SECONDARY ANALYSES

To examine the impact of “dose,” the group with parental PTSD was further subdivided based on the number of parents with PTSD. **Figure 2A** shows that there were no differences in cortisol levels among offspring having either 1 or 2 parents with PTSD. When the offspring group with parental PTSD was further subdivided based on the sex of the only affected parent, offspring with paternal PTSD only were not significantly different in mean cortisol level than offspring with no parental PTSD or comparison subjects (Figure 2B). Mean cortisol levels were similar for offspring with PTSD in both parents and those with maternal PTSD only, whereas both groups differed from offspring with no parental PTSD ($P = .02$ and $P = .045$, respectively) and from comparison subjects ($P = .009$ and $P = .02$, respectively).

Regression analysis was performed to further examine the secondary analysis findings. After controlling for BMI, a current depression or anxiety diagnosis, and CAPS-L lifetime total score in regression analysis, there were significant negative partial correlations between mean cortisol level and the presence of maternal ($r_{44} = -0.41$; $P = .004$) and paternal ($r_{44} = -0.32$; $P = .03$) PTSD. However, after additionally controlling for the presence of PTSD in the other parent, only maternal PTSD retained its significant association with offspring mean cortisol levels (for paternal PTSD: $r_{43} = -0.21$; $P = .17$; for maternal PTSD: $r_{43} = -0.34$; $P = .02$).

RELATIONSHIP BETWEEN CORTISOL LEVELS AND SEVERITY OF PARENTAL PTSD AND CTQ SCORES

Because there was variability in the severity of parental PTSD (parental PTSD and non-parental PTSD groups: range, 13-65 and 3-33, respectively), we also analyzed these dimensional measures of parental PTSD symptoms. Partial correlational analyses were performed using mean cortisol levels and dimensional PPQ scores (controlling for BMI, mood or anxiety disorder, and PTSD severity) as an alternative to dichotomizing survivors with and without PTSD. When the whole sample was considered, there was a significant association between mean cortisol levels and severity of parental PTSD ($r_{42} = -0.41$; $P = .006$) that was reduced when only Holocaust offspring were considered ($r_{26} = -0.39$; $P = .04$) and was further reduced to nonsignificance when examined in the smaller offspring subgroup with parental PTSD ($r_{16} = -0.36$; $P = .14$). There were no significant associations between mean cortisol levels and total CTQ scores in the whole sample ($r_{33} = -0.15$; $P = .40$), the offspring-only group ($r_{22} = -0.21$; $P = .32$), or the subgroup of offspring with parental PTSD ($r_{13} = 0.02$; $P = .95$).

RELATIONSHIP BETWEEN CORTISOL LEVELS AND YEARS BORN AFTER TRAUMA

The relationship between mean cortisol levels and the number of years from the end of the Holocaust to the birth of the offspring was examined, controlling for BMI, current age, and mood or anxiety disorder, including PTSD. Lower cortisol levels were seen in offspring born in closer proximity to the Holocaust ($r_{28} = 0.37$; $P = .04$).

COMMENT

Herein we show that plasma cortisol levels are lower in the adult children of Holocaust survivors than in matched controls at several times across a 24-hour cycle. This effect is confined to the offspring of Holocaust survivors with PTSD and in correlational analysis was related to the severity of parental PTSD. Offspring with parental PTSD also demonstrated changes in some chronobiological parameters previously identified as altered in trauma survivors with PTSD²⁰ despite that no subject had PTSD at assessment. However, the overall pattern of alterations observed in the offspring with parental PTSD did not follow that reported for PTSD, allowing differentiation between parameters associated with risk vs those associated with PTSD pathogenesis.

Specifically, consistent with our previous observation in combat veterans with PTSD,²⁰ offspring with parental PTSD showed a lower cortisol mesor, reflecting reduced glucocorticoid production during the 24-hour cycle. However, offspring with parental PTSD also showed reduced cortisol amplitude. Consequently, the circadian quotient, reflecting the amplitude-mesor ratio, was not different among the 3 groups. In contrast, a higher circadian amplitude and a greater amplitude-mesor ratio were reported in combat veterans with PTSD compared with

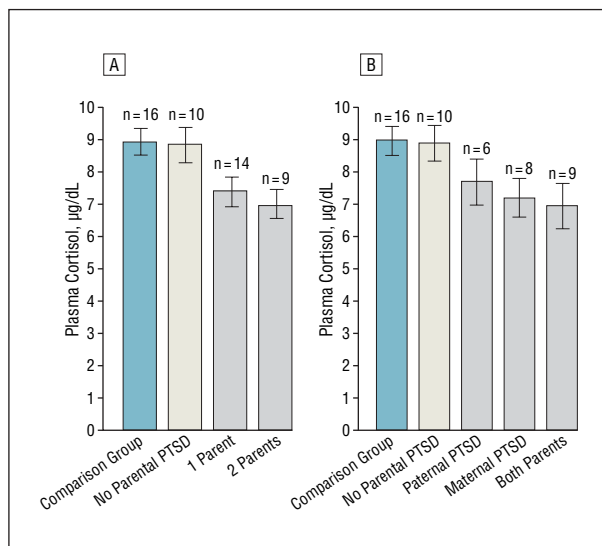


Figure 2. Effect of parental posttraumatic stress disorder (PTSD) on secondary analyses. Data are given as estimated means. Error bars represent SE. A, Holocaust offspring with parental PTSD are subdivided according to the number of parents with PTSD. The significant effect of group ($F_{3,42} = 3.88$; $P = .02$; partial $\eta^2 = 0.22$) was a result of having either 1 (mean \pm SE cortisol level, 7.39 ± 0.44 µg/dL) or 2 (6.97 ± 0.56 µg/dL) parents with PTSD vs having no parents with PTSD (ie, being the offspring of a survivor without PTSD) (8.84 ± 0.52 µg/dL) or being a nonexposed comparison subject (8.92 ± 0.42 µg/dL). There was a main effect of time of day ($F_{12,40,520.88} = 2.64$; $P = .002$; partial $\eta^2 = 0.06$). In addition, body mass index ($F_{1,42} = 8.81$; $P = .005$) and a current depression or anxiety diagnosis ($F_{1,42} = 8.56$; $P = .006$) were significant covariates. B, Holocaust offspring with parental PTSD are subdivided according to the sex of 1 parent with PTSD. Offspring with PTSD in both parents (mean \pm SE cortisol level, $6.95 \pm .57$ µg/dL) were comparable with those with maternal PTSD only ($7.16 \pm .61$ µg/dL) and different from offspring with paternal PTSD only ($7.69 \pm .68$ µg/dL), offspring with no parental PTSD ($8.86 \pm .53$ µg/dL), and comparison subjects ($8.93 \pm .42$ µg/dL). There were significant main effects of group ($F_{4,41} = 2.94$; $P = .03$; partial $\eta^2 = 0.22$) and time of day ($F_{12,64,518.04} = 2.48$; $P = .003$; partial $\eta^2 = 0.06$). Body mass index ($F_{1,41} = 8.43$; $P = .006$) and a current depression or anxiety diagnosis ($F_{1,41} = 8.74$; $P = .005$) were significant covariates. To convert cortisol to nanomoles per liter, multiply by 27.588.

healthy and depressed patients.²⁰ Thus, reduced glucocorticoid output may be a relatively stable risk factor for PTSD, but chronobiological parameters may vary flexibly to adjust to environmental demands.

Although the sample sizes were relatively small, significance was achieved in the comparisons of offspring with parental PTSD with each of the other groups. However, the lack of significance and the low power ($1 - \beta = .03$) of the comparison of offspring without parental PTSD with controls is not principally due to the small sample sizes of these groups because the effect size of this comparison is only 0.05.

In contrast, sample sizes for the secondary analyses by parental number and sex should be interpreted cautiously given the small samples resulting from this subdivision. Despite the small samples, we performed such analyses to better understand the nature of the putative acquired cortisol levels and patterns. Having 1 vs 2 parents with PTSD had similar effects on offspring cortisol levels. However, low cortisol levels seemed to be more strongly associated with maternal-only than paternal-only PTSD. This result is supported by analysis of a continuous measure of parental PTSD symptom severity, in which offspring cortisol level was significantly associ-

ated with maternal PTSD symptoms after controlling for paternal PTSD symptoms, whereas paternal PTSD symptoms were not significant after controlling for maternal PTSD symptoms.

A major limitation of this study is that offspring provided the information pertaining to parental PTSD symptoms on which group distinctions were based. Although we previously established that there is strong agreement between offspring ratings of parental PTSD and independent physician or psychologist ratings of the parent,²¹ if offspring cortisol levels were associated with their perception of parental PTSD, this could lead to a circularity of inference. Thus, if low cortisol levels reflect offspring characteristics such as irritability and emotional withdrawal, or other psychopathologic conditions, this might result in a greater projection of analogous parental characteristics so that "parental" PTSD was an expression of offspring characteristics. To protect against this possibility, offspring PTSD symptoms and diagnosis of other mood and anxiety disorders were controlled for in all the analyses investigating cortisol effects.

Another possible source of circularity is that low cortisol levels could increase offspring sensitivity to parental symptoms so that individuals with low cortisol levels would be better at detecting PTSD when it occurred. With respect to the difference in offspring cortisol levels based on maternal vs paternal PTSD, because the mother was most likely the primary caregiver, such misattribution might disproportionately affect paternal PTSD ratings. If PTSD ratings were largely based on the extent of parental contact, having more contact with mothers would be reflected by substantially higher PPQ scores in mothers. However, in the present study there were no significant differences between PPQ severity in mothers or fathers. Thus, this type of attributional bias does not seem to explain the observed differences between maternal vs paternal PTSD in offspring cortisol levels.

That offspring of Holocaust survivors with PTSD have previously been found to report greater childhood emotional abuse and neglect than offspring of Holocaust survivors without PTSD or demographically similar controls provides some evidence of environmental mediation of parental PTSD effects.¹³ However, the current findings are not fully explained by such exposures because the offspring of survivors with and without parental PTSD did not differ significantly in CTQ scores, and neither were cortisol levels correlated significantly with CTQ scores. This lack of association does not rule out the effect of other environmental factors, including those associated with the impact of the parental care environment; however, the recent demonstration of lower cortisol levels in year-old infants born to mothers who developed PTSD as a result of direct exposure to 9/11 compared with infants born to similarly exposed mothers who did not develop PTSD implies that such effects would occur early in life. Possibly, important environmental alterations may occur in utero as evidenced by the fact that effects on infant cortisol levels depended on the trimester of the mother on 9/11.¹⁷

Alterations in glucocorticoid receptor (GR) sensitivity that are known to occur very early during the postnatal period, possibly coinciding with the period in which

normal maternal infant attachments are developing, may be relevant to the present findings.²⁷ The activity of genes regulating HPA axis activity can be programmed by early life events and, specifically, by nuances related to maternal care.²⁸ Maternal behaviors, which alter the infant's environment, can, in turn, result in long-lived changes in hippocampal GR expression and HPA function that are subsequently transmitted intergenerationally.²⁹ The GR gene is particularly sensitive to postnatal "programming" because it has complex tissue-specific promoters that are susceptible to epigenetic modification.³⁰ Modifications of these promoters during development can alter the "set point" of receptor function in feedback sites and, hence, glucocorticoid secretion.³¹

The maternal behaviors of increased licking and grooming induced by "early handling" are widely interpreted as beneficial, as are their biological outcomes, in the adult offspring rat.³² It is, therefore, interesting that the epigenetic modifications following such paradigms result in HPA alterations in the same direction as those described in PTSD (eg, increased GR sensitivity, enhanced cortisol [corticosterone] suppression after dexamethasone administration, and lower ambient glucocorticoid levels).² Possibly, mothers with PTSD form attachments to infants marked by enhanced attention and contact (ie, rather than relative neglect or physical abuse). Indeed, adult Holocaust offspring presenting for psychotherapy characteristically complain of difficulties in physically and emotionally separating from parents, implying strong attachments, although not attachments that protect from the risk of developing psychopathologic disorders. Alternatively, the lowered basal cortisol levels in Holocaust offspring with parental PTSD may be more similar to the low cortisol levels observed in offspring of monkeys reared by mothers subjected to variable high-stress foraging conditions, conditions that are associated with more negative outcomes in monkeys.³³

Parental PTSD may confer risk of PTSD in the offspring by affecting the predisposition to a modification that may later affect the response to a traumatic event. If what gets "programmed" is the set point of glucocorticoid secretion and an enhanced capacity for responsiveness of the HPA axis, this would explain why cortisol levels can remain low in the face of other chronobiological adjustments in patterns of release.

Epigenetic changes in the GR gene similar to those induced postnatally by maternal behavior can occur prenatally via glucocorticoid exposure.^{34,35} Permanent effects of elevations in maternal glucocorticoid levels during pregnancy resulting from stress or undernutrition are thought to be responsible for the development of subsequent cardiometabolic disease^{36,37} and psychopathologic conditions in offspring.^{38,39} Whether the intense nutritional challenge and mortal stress of the Holocaust affected the present findings is unclear but cannot be ruled out. Nutritional challenge was certainly not a factor in low cortisol levels in offspring with maternal PTSD after 9/11. That lower cortisol levels in offspring were associated with the proximity of the pregnancy to the time of Holocaust exposure in this study and with trimester of trauma exposure in the 9/11 study implies that the maternal environmental effect depends on critical periods

of glucocorticoid programming, trauma exposure, and the presence of the state or trait of PTSD.

The present findings do not rule out a genetic interpretation, largely because the present data are inconclusive regarding the differential effects of maternal vs paternal PTSD. However, classic genetic mechanisms would not support an explanation in which cortisol effects were more determined by maternal PTSD, and neither would they explain the waning of the effect of low cortisol levels in offspring as the time increased between the Holocaust and maternal pregnancy. Instead, in that the present findings are not explained by the presence of offspring psychopathologic disorders or CTQ scores, it is possible to speculate that they represent manifestations of developmental programming by environmental effects occurring after birth. One plausible scenario may be that severe trauma affects or conditions maternal physiologic features, or the biological features of the unfertilized oocytes or maternal uterine environment, to a state that determines a set point for programming of glucocorticoid responsivity in the offspring. The added effects of the mother's PTSD, driven by variations in maternal care, could further facilitate HPA axis alterations in the postnatal period. Alternatively, an imprinted or sex-delimited gene may be the first risk factor for PTSD and attendant neuroendocrine markers (for mothers and offspring), and the environmental challenge (eg, Holocaust exposure of oocytes or direct effects on the uterus) would act as the instigating factor on an already vulnerable background. Although these putative mechanisms are speculative, they can be examined by determining the relative contributions of maternal and paternal biological factors to second- and third-generation offspring.

In conclusion, the present findings demonstrate that low cortisol levels in offspring are associated particularly with maternal PTSD. A possible mechanism for this association is transmission via early glucocorticoid programming. In such offspring, trauma exposure may increase the probability of PTSD and further alter chronobiological parameters in a manner that is distinguished from HPA alterations in other psychiatric disorders. That some biological manifestations of PTSD represent traits that may predate trauma exposure provides an important context for interpreting studies of the neural correlates of fear in PTSD. Accordingly, the findings imply that in some cases it may be necessary to supplement current approaches focused on reliving the index trauma with interventions that address preexisting risk factors. Although the implications for PTSD prophylaxis cannot be specified from these results, they have clear clinical applications, including assessment of parental PTSD in patients with PTSD and evaluation of stressful events during pregnancy and early childhood. Indeed, the data suggest that examination of epigenetic or in utero phenomena should be added to the search for genetic polymorphisms that may underlie individual differences that increase vulnerability to this disorder.

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