Abstract

We have previously demonstrated lower mean 24-h urinary cortisol excretion in adult offspring of Holocaust survivors with parental posttraumatic stress disorder (and lifetime PTSD), compared to offspring without parental PTSD, and to demographically similar comparison subjects. In the current study, we re-analyze data from our previously published report, plus four new subjects, to further examine the relationship between cortisol and severity of PTSD symptoms in offspring and their parents. We also examine the contribution of current depressive disorder to cortisol levels. Two-way analysis of variance revealed lifetime PTSD to be associated with significantly lower cortisol levels, while depressive disorder was associated with higher cortisol levels. The presence of parental PTSD was associated with lower cortisol excretion in the offspring only if both parents were affected. There were significant negative correlations between severity of parental PTSD and offspring urinary cortisol excretion, and between severity of offspring PTSD symptoms and urinary cortisol levels. The findings amplify our earlier descriptions of children of Holocaust survivors with PTSD as a sample ‘at risk’ for PTSD by demonstrating relationships between lowered cortisol excretion in these offspring and their experience of their parents’ PTSD symptoms. Published by Elsevier Science Ltd.

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1. Introduction

The idea that there are individual differences in responses to stressful environmental events (e.g. Shalev and Yehuda, 1998) has constituted a pivotal advance in understanding how similar experiences can result in different psychopathological outcomes. That intergenerational effects may contribute to these inter-individual differences provides the relevant background for this study. Indeed we (Yehuda et al., 1998a) and others (Solomon et al., 1988) have demonstrated that adult children of Holocaust survivors have a greater prevalence of lifetime posttraumatic stress disorder (PTSD), as well as other types of psychopathologic outcomes, compared to demographically similar persons who have experienced equivalent types of Diagnostic and Statistics Manual-IV (DSM-IV) traumatic events. We further showed that PTSD in children of Holocaust survivors appeared to be strongly related to parental PTSD, but not to other parental psychiatric disorders (Yehuda et al., 1998b). Based on these observations we have suggested that adult offspring of Holocaust survivors constitute an ‘at risk’ group for PTSD. These findings led us to initiate biologic investigations in adult children of Holocaust survivors, focusing primarily on putative biologic markers of risk for PTSD (Yehuda et al., 2000).

We recently reported that low 24-h urinary cortisol excretion in offspring was significantly associated with both lifetime PTSD and with parental PTSD, whereas having diagnosis of anxiety or depressive disorder was associated with relatively higher cortisol levels (Yehuda et al., 2000). We suggested that our findings might indicate the transgenerational transmission of stress response characteristics, similar to those that have been demonstrated in animal models of early handling (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999). Specifically, the early effects of maternal behavior in rat pups have recently been shown to persist across multiple generations (Francis et al., 1999), and to be associated with increased hippocampal glucocorticoid receptor expression similar to that proposed in PTSD (Yehuda et al., 1995).

In the present study, we reanalyzed recently published data (with the addition of four subjects since our previous publication) of mean urinary 24-h cortisol excretion in offspring of Holocaust survivors, in order to examine more closely the association between maternal and paternal PTSD and offspring cortisol levels. In the recently published study we defined offspring as having been raised by at least one biological parent who survived the Nazi Holocaust, and considered offspring to have the risk factor of parental PTSD if at least one parent met the diagnostic criteria for PTSD (Yehuda et al., 2000). However, we did not distinguish whether the parental PTSD occurred in one or both parents, nor did we further assess specific associations of parental PTSD symptomatology to cortisol excretion in offspring.

In the current analyses we conducted a more detailed examination of the relationship between parental PTSD and cortisol excretion in the offspring. We divided the previous sample into three groups: those with no parental PTSD, offspring with maternal or paternal PTSD, and offspring with both maternal and paternal PTSD. In addition, we examined correlations between 24-h urinary cortisol excretion in offspring and severity of PTSD symptoms in parents and offspring.
2. Methods

The study was approved by the Institutional Review Board of the Mount Sinai School of Medicine. All subjects provided written informed consent prior to their participation. The sample consisted of 39 offspring (seven men and 32 women), and 15 healthy comparison subjects (eight men and seven women) between the ages of 26 and 61 years. Recruitment for the study was as previously described (Yehuda et al., 2000). Exclusion criteria for all subjects included significant current alcohol and/or substance abuse, or any history of psychotic disorder or bipolar illness. Comparison subjects were Jewish, and within the same age range as offspring participants, and free from current psychiatric diagnoses.

Medical information was obtained for all participants, using a physician-reviewed medical checklist. Individuals were excluded from the study if they had an active major medical condition, or if they had been treated with beta-blockers, lithium, or other psychotropic medications within two months of the study. Subjects were not withdrawn from medications to participate in this protocol and a list of medications used by the original sample of 50 is presented in our previous paper (Yehuda et al., 2000). Of the four additional subjects, one female was taking estrogen replacement at the time of testing and the other three were unmedicated.

PTSD symptoms were assessed using the Clinician Administered PTSD Scale (CAPS) following DSM-IV format and administered by a trained clinician (Blake et al., 1990). Other psychiatric diagnoses were also made by the same clinician using the Structured Clinical Interview for the DSM-IV (SCID) (Spitzer et al., 1995) or, for subjects enrolled prior to the introduction of the DSM-IV, for the DSM-III-R (Spitzer and Williams, 1987), as previously described (Yehuda et al., 2000).

Parental PTSD was rated by the offspring using a scale developed at the Traumatic Stress Studies Program (Yehuda et al., 2000). This instrument, the Parental PTSD Scale, is administered as a self-report measure which first inquires about the nature of each parent’s Holocaust-related experiences (i.e. concentration camp, ghetto, hidden, etc.) and then asks the subject to complete a checklist based on the 17 DSM-IV symptoms of PTSD for each parent. The offspring were asked to estimate the average severity of each of the parents’ symptoms on the basis of recall from their childhood, adolescence, and early adult years. PTSD symptoms were rated on a four-point Likert-type scale that had anchors similar to those on the CAPS. Although the accuracy of an adult offspring’s estimate of the actual extent of parental PTSD symptoms is difficult to ascertain, the questionnaire provided an index of the subjective perception of the parents’ symptoms. In 11 subjects, we were able to compare the results of this questionnaire with the results of CAPS scores from parents that we had interviewed directly. With one exception, raters and offspring came to the same conclusion regarding the presence or absence of parental PTSD, and general level of severity. In the case of the single discrepancy, clinical data suggested that both parents had had lifetime PTSD, whereas the adult child perceived only her father to have suffered from PTSD. For these analyses, the adult offspring’s impression was used rather than the clinician rated CAPS data. In three cases, estimates of severity
of parental PTSD were made directly from their CAPS data due to missing or incomplete responses by the offspring.

A short screen for parental exposure to traumatic events (e.g. combat, natural disaster, physical or sexual assault) was administered to comparison subjects in order to estimate the likelihood of parental PTSD in this group. None of the participants in this group reported parental exposure to trauma on this measure.

Urine was collected for 24 h after the first voided urine following awakening, including the first voided urine on the following day. Two-liter polyethylene collection bottles were used and kept in freezers in the subjects’ residences in order to ensure stability of cortisol. Collections were scheduled to occur on days that were anticipated not to be particularly stressful, in order to obtain samples that would reflect basal secretion. Typically, subjects planned to be at home for the 24-h period in order to facilitate collection. Urinary-free cortisol levels were determined using an extraction procedure and radioimmunoassay kit from Clinical Assays, Inc. (Cambridge, MA) (interassay coefficient of variation=4.0).

Group comparisons were carried out using Student’s t-tests or analysis of variance (ANOVA). Post hoc contrasts used Tukey’s Honestly Significant Difference test, or Dunnett’s T3 where variances were unequal. Chi-square tests were used for comparisons of categorical data. Linear associations were assessed using Pearson’s correlation coefficient and partial correlation.

3. Results

3.1. Sample characteristics

The offspring group was significantly older (offspring mean=41.62 yrs, SD=0.73; comparison mean=33.00 yrs, SD=7.92, t(52)=3.64, P<0.001) and had a higher proportion of females relative to comparison subjects (χ²=6.76, df=1, P<0.009). However, cortisol was not significantly correlated with age in this sample (Pearson’s r=0.000; df=54; ns), and no difference in 24-h urinary cortisol levels was apparent for males vs. females (t(52)=0.76, ns). Therefore, neither age nor gender was controlled for in subsequent analyses. Offspring and controls did not differ in urinary volume (t(49)=−0.37; ns) or urinary cortisol concentration (t(49)=1.81, ns).

None of the 15 comparison subjects had a history of PTSD or a current Axis 1 psychiatric diagnosis; two had a history of major depression, and one had past social phobia. Among the 39 offspring, 13 (33%) met criteria for lifetime PTSD. Of these, 10 (77%) had at least one current comorbid diagnosis (major depression, N=6; dysthymia, N=2; generalized anxiety disorder, N=2; panic disorder, N=2; bulimia, N=1; adult attention deficit disorder, N=1). Of the 26 offspring without PTSD, 10 (38%) met criteria for at least one other Axis I disorder (dysthymia, N=3; depressive disorder, NOS, N=m1; generalized anxiety disorder, N=4; panic disorder, N=2; phobias, N=2; adjustment disorder, N=2). The incidence of other psychiatric diagnoses was higher among those offspring with PTSD than those without (χ²=7.01, df=1,
There was a higher incidence of PTSD among offspring who had a parent with PTSD compared to offspring without parental PTSD ($\chi^2=9.75$, df=1, $P=0.002$). For the entire group of offspring, those with parental PTSD showed a non-significant trend also to have a higher incidence of other psychiatric diagnoses than those without parental PTSD ($\chi^2=3.28$, df=1, $P<0.07$). However, when this analysis was repeated for the subgroup of offspring without their own PTSD ($N=26$), there was no difference in the incidence of other psychiatric diagnoses between offspring with and without parental PTSD (46% versus 31%, $\chi^2=0.65$, df=1, ns). This suggests that, in this small sample, the increased frequency of psychiatric diagnoses in the offspring with parental PTSD is a reflection of comorbidity with the offspring’s own PTSD, and not of not parental PTSD.

### 3.2. Cortisol comparisons

With the current sample of 39 offspring, mean 24-h urinary cortisol excretion was no longer significantly lower than in controls ($F(1,52)=1.69$, $P<0.20$), as previously reported (Yehuda et al., 2000). This was due, in part, to the fact that three of the four newly added subjects met criteria for depressive disorder, and accordingly had cortisol levels at the high end of the distribution. Given that cortisol levels have been found to be directionally different in subjects with depressive disorder and PTSD (Holsboer, 2000; Catalan et al., 1998; Yehuda, 1998), we assessed the contribution of depressive disorder (i.e. major depressive disorder or dysthymia) to the variance in urinary cortisol excretion.

Two-way ANOVA of the sample as a whole, in which both presence or absence of lifetime PTSD and presence or absence of current major depressive or dysthymic disorder were entered as separate factors, demonstrated significant main effects on 24-h urinary cortisol for both PTSD ($F(1,50)=15.15$, $P<0.001$) and depressive disorder ($F(1,50)=6.06$, $P<0.02$), but no significant interaction between the two ($F(1,50)=1.29$, ns). Fig. 1 shows that while PTSD is associated with lower cortisol levels, depressive disorder appears to be associated with elevated cortisol, particularly in the absence of concurrent PTSD.

Given that the influence of depressive disorder on cortisol excretion appears to be in the opposite direction to that of PTSD, analyses of parental influences on offspring urinary cortisol levels were conducted accounting for the presence of current major depression or dysthymia in the offspring. As in our prior report (Yehuda et al., 2000), the presence of parental PTSD was associated with lower cortisol. A two-way ANOVA found a significant main effect of parental PTSD on 24-h urinary cortisol ($F(1,50)=5.44$, $P<0.03$). Depressive disorder, as the second factor, did not quite reach statistical significance ($F(1,50)=3.74$, $P<0.06$).

When the sample was further divided based on whether neither, one or two parents had PTSD, two-way ANOVA again found a significant main effect for group ($F(2,48)=5.47$, $P<0.007$). Participants who had two parents with PTSD had the lowest cortisol levels. Post hoc tests showed significantly lower cortisol levels in offspring...
spring who had two parents with PTSD compared to both offspring who had only one parent with PTSD (Dunnett’s T3, $P<0.03$) and those with no parental PTSD (Dunnett’s T3, $P<0.002$). Urinary cortisol values for individuals who had only one parent with PTSD did not differ from those for individuals with no parental PTSD. There was a trend for a main effect of depressive disorder on cortisol levels ($F(1,48)=3.90$, $P=0.06$). Fig. 2 provides a graphic representation of the cortisol data of subjects divided on the basis of having one or both parents with PTSD, and additionally demonstrates that there were no group differences in cortisol excretion based on whether the single parent with PTSD was the mother or the father.

### 3.3. Correlational analyses

Partial correlations within the offspring group revealed significant associations between offspring urinary cortisol excretion and parental PTSD symptoms, controlling for depressive disorders in the offspring. Offspring cortisol levels were significantly associated with the sum of PTSD symptom severity scores for mothers and fathers combined (partial $r=-0.402$, df=35, $P<0.02$). This correlation reflects a particularly high association between the intrusive subscale (partial $r=-0.48$, df=35, $P<0.003$), and a more modest, but significant association between scores on the hyperarousal subscale (partial $r=-0.358$, df=35, $P<0.04$). Scores on the avoidance subscale were not significantly associated with cortisol levels in offspring (partial $r=-0.26$, df=35, ns).

The magnitude of the correlations between offspring cortisol and parental PTSD
is impressive, particularly when compared to the equivalent correlations between cortisol and the offspring’s own PTSD symptoms, controlling for offspring depressive disorders. Scores for the clinician-rated CAPS were associated with 24-h urinary cortisol in the offspring as follows: for total CAPS score, partial $r=-0.47$, df=36, $P<0.003$; for the intrusive subscale, partial $r=-0.45$, df=36, $P<0.005$; for hyperarousal $r=-0.45$; df=36; $P<0.004$; and for avoidance, partial $r=-0.43$, df=36, $P<0.008$. Thus, with the exception of a greater correlation of cortisol with avoidance symptoms in the offspring, 24-h urinary cortisol levels in the offspring appeared to be associated with PTSD symptoms in their parents as much as with their own PTSD symptoms. Fig. 3 shows the correlations between offspring urinary cortisol excretion and both parental intrusive symptoms (panel A) and their own lifetime intrusive symptom severity (panel B).

4. Discussion

Several points are highlighted by the re-analyses of these data. First, with the addition of even a few subjects, the previous finding of an overall group difference between offspring of Holocaust survivors and demographically-similar controls in 24-h urinary cortisol excretion no longer reaches statistical significance. This is in large part due to the presence of depressive disorders, and associated higher cortisol levels, in three of the added subjects. In the prior paper, we reported a significant difference in 24-h urinary cortisol excretion in offspring with and without current psychiatric disorder, including depression as well as other anxiety disorders. How-
ever, offspring with current psychiatric disorder did not show differences in urinary cortisol levels compared to controls. In the current analyses, we specifically considered current depressive disorders as a potentially significant confound. The dual and opposing main effects of PTSD and depression on cortisol levels in this sample further underscore the importance of considering current depression in neuroendocrine studies of PTSD. Of note, it appears that cortisol levels may not be as elevated in persons with current depressive disorder who also have lifetime PTSD. Thus, the findings also indirectly suggest a possible explanation for some of the variance in cortisol levels found in studies of patients with an affective disorder (i.e. the possibility of lifetime and/or concurrent PTSD).

The differences in cortisol in PTSD patients with and without comorbid depression suggest that different regulatory mechanisms may be operating. Depression appears to involve the dysregulation of several biologic systems, manifest by both high and low levels of hormones and other neuroregulators, consistent with a biological desensitization (Siever and Davis, 1986; Yehuda et al., 1996). In contrast, in PTSD there appears to be a more tightly regulated pattern of hormonal release and response, reflecting biological sensitization (Yehuda et al. 1996, 1995). The results of this study suggest that the regulatory patterns that confer risk for a disorder such as PTSD may nonetheless undergo subsequent change in response to a superimposed psychiatric illness (e.g. depressive disorder). The current findings suggest that several specific patterns of biologic alterations may co-exist and may be regulated simultaneously in competing directions. Thus, even biologic indices of risk may be altered by subsequent state-related influences.

Second, while parental PTSD is associated with decreased mean 24-h urinary cortisol excretion in offspring, in this small sample we could only detect noticeable differences in cortisol in those who rated both parents as having PTSD. This might reflect a protective effect of having one unaffected parent during development. The
emerging animal literature on the role of maternal experiences on offspring stress responses (Francis et al., 1999) reinforces the need to consider environmental and developmental influences in relation to vulnerability. The current study did not address this issue.

While it is important to maintain caution in interpreting these preliminary findings, the robustness of the correlations between cortisol excretion in the offspring and parental PTSD symptoms is impressive. Because estimates of parental PTSD symptom severity were provided by the offspring they may, in part, reflect the offspring’s own attributions and projections. However, in the context of examining vulnerability, this attribution may provide an important insight. Conceivably, it is the obvious distress of the afflicted parent(s) that confers risk to the offspring for PTSD or other disorders associated with lowered cortisol (e.g. chronic pelvic pain; Heim et al., 1998). Indeed, this may explain why the more visible intrusive and hyperarousal symptoms were more highly correlated with offspring cortisol excretion than were the avoidance symptoms, which may be more difficult to perceive in another. Parental avoidance symptoms were not significantly correlated with cortisol levels in the offspring. These results point to the need for larger sample sizes so that more subtle relationships can be discerned.

Although the experiences of Holocaust survivors are unique, the current findings may speak more generally to the experiences of children of trauma survivors. In considering the impact of parental PTSD as a risk factor, it is important to acknowledge that the quality of parenting given by an individual with chronic PTSD may be fundamentally altered by the disorder. Specifically, the presence of parental PTSD may be associated with other known risk factors, such as an environment in which a child may be emotionally abandoned, abused, or physically neglected or threatened. Thus, parental PTSD may result in a behavioral cascade, leading to childhood trauma and vulnerability to PTSD in the offspring. Certainly, the cycle of trauma and stress response may be perpetuated through familial relationships, and the observed low cortisol may not only be a reflection of PTSD in the offspring, but of transgenerational continuity in this disorder.

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**References**