Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors

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Abstract

Objective—In animal models, early life exposure to major environmental challenges such as malnutrition and stress results in persisting cardiometabolic, neuroendocrine and affective effects. While such effects have been associated with pathogenesis, the widespread occurrence of 'developmental programming' suggests it has adaptive function. Glucocorticoids may mediate 'programming' and their metabolism is known to be affected by early life events in rodents. To examine these relationships in humans, cortisol metabolism and cardiometabolic disease manifestations were examined in Holocaust survivors in relation to age at exposure and affective dysfunction, notably lifetime posttraumatic stress disorder (PTSD).

Methods—51 Holocaust survivors and 22 controls without Axis I disorder collected 24-hr urine samples and were evaluated for psychiatric disorders and cardiometabolic diagnoses. Corticosteroids and their metabolites were assayed by gas chromatography mass spectroscopy (GCMS); cortisol was also measured by radioimmunoassay (RIA).

Results—Holocaust survivors showed reduced cortisol by RIA, and decreased levels of 5α-tetrahydrocortisol (5α-THF) and total glucocorticoid production by GCMS. The latter was associated with lower cortisol metabolism by 5α-reductase and 11β-hydroxysteroid dehydrogenase (11β-HSD) type-2. The greatest decrements were associated with earliest age of Holocaust exposure and less severe PTSD symptomatology. Cardiometabolic manifestations were associated with decreased 11β-HSD-2 activity. In contrast to the relationship in Holocaust survivors, in controls, 5α-reductase was positively associated with trauma-related symptoms.

Conclusion—Extreme malnutrition and related stress during development is associated with long-lived alterations in specific pathways of glucocorticoid metabolism. These effects may be adaptive and link with lower risks of cardiometabolic and stress-related disorders in later life.

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Keywords

Posttraumatic stress disorder; Glucocorticoid metabolism; Biological markers; 5α-tetrahydrocortisol; 11 β-hydroxysteroid dehydrogenase; Child psychiatry

INTRODUCTION

Exposure to extreme malnutrition and/or stress during critical prenatal or postnatal developmental 'windows,' is associated with persisting alterations in psychological and physiological parameters in adult life, a phenomenon dubbed 'developmental programming' (Barker, 1998). In animal models, stress, glucocorticoid overexposure or undernutrition cause permanent changes in cardio-metabolic, neuroendocrine and behavioral functions that are mediated, in part, by changes in offspring glucocorticoid metabolism (Seckl & Meaney, 2004). To date, there has been an emphasis on detrimental effects, however, it is becoming increasingly recognized that some consequences of extreme stress may reflect anticipatory adaptations to projected demands (Gluckman & Hanson, 2004).

There appear to be critical developmental windows for such effects. For example, adult offspring of women who were pregnant during the Dutch Hunger Winter of 1944/45 show increased rates of cardiovascular disease and schizophrenia if their mothers were exposed in early gestation (Susser & Lin, 1992; Painter et al, 2006), but decreased glucose intolerance if exposure was in later gestation (Ravelli et al, 1998). Infants of women who developed PTSD as a result of being in or near the World Trade Center on 9/11/2001 during pregnancy had reduced salivary cortisol levels if maternal exposure on 9/11 occurred in the last trimester of pregnancy (Yehuda et al, 2005a). Such data suggest that the timing in development during which challenge occurs may define the nature of persistent effects. Early postnatal environmental factors also exert programming effects in rodents and humans (Kaffman & Meaney, 2007). Again, offspring glucocorticoid parameters are frequently affected (Phillips, 2007).

In contrast to major depression in which cortisol levels are often elevated, PTSD has been associated with comparatively lower stress-induced cortisol levels and, less consistently, with reduced integrated levels of basal urinary cortisol (Yehuda, 2002; Young & Breslau, 2004). Lower urinary cortisol has been reported in elderly Holocaust survivors and combat veterans with PTSD, groups which have, *inter alia*, a significantly higher prevalence of early childhood adversity/abuse (Yehuda, 2002; Yehuda et al, 2005b). Reduced or increased cortisol levels have also been observed in adult survivors of severe child abuse without PTSD though the contribution of other affective disorders complicates interpretation (Debellis et al, 1994; Heim et al, 2000; Heim et al, 2001; Bremner et al, 2003).

Here we examine whether the previously observed discrepancies in cortisol levels among Holocaust survivors with and without PTSD reflect underlying differences in glucocorticoid metabolism following early life challenge, associate with age at exposure and examine these effects in relation to affective and cardiometabolic diagnoses. Tissue-specific metabolism of glucocorticoids contributes to their cellular actions and hypothalamic-pituitary-adrenal (HPA) axis function (Yau et al, 2001). Key enzymes such as the 11β-hydroxysteroid dehydrogenases (11β-HSD), that catalyze the interconversion of active cortisol and inert cortisone (Seckl et al, 2001), and 5α- and 5β-reductases (Westerbacka et al, 2003), are associated with cardiometabolic as well as central nervous system (CNS) functions (Andrew et al, 1998). Changes in these enzyme systems alter the effective half-life of cortisol and may accordingly influence the regulation of the HPA axis (Andrew et al, 1998). Even relatively small changes in regional peripheral metabolism, if sustained chronically, could influence both local tissue

exposure to cortisol and HPA axis dynamics (Dannan et al, 1986; Paterson et al, 2007), thus determining individual risk for cardiometabolic and psychiatric disorders.

METHOD

Subjects

Fifty-one Holocaust survivors (age range 60-89) and 22 age-comparable Jewish adults (age range 55-85) unexposed to the Holocaust were recruited to participate in a diagnostic interview and to collect urine over a 24-hr period at home. For this research, 'Holocaust survivor' was defined as being Jewish, and having lived in Nazi occupied Europe between 1939 and 1945, either in a concentration camp, ghetto or in hiding. Participants were excluded if they had a history of psychosis, bipolar disorder, alcohol or substance dependence, organic mental disorder (including stroke) or dementia. Non-exposed participants were free of any lifetime Axis I disorder. PTSD diagnoses and severity ratings were made using the Clinician Administered PTSD Scale (CAPS) (Blake et al, 1995). Other psychiatric diagnoses were confirmed using the Structured Clinical Interview for DSM-IV (Spitzer et al, 1995). The Mississippi Scale (Lauterbach et al., 1997) was used to assess trauma-related symptomatology in both survivors and comparison subjects, as it provides a broad index of the impact of a traumatic exposure on an individual's life and does not rely on the presence or absence of criterion A events for its completion.

Subjects completed an extensive survey of current medical illnesses or conditions, medical symptoms, and all current medications that was reviewed by a physician. Persons taking oral corticosteroids or medications known to interfere with the HPA axis were excluded (Yehuda, 2002). Three of 73 were taking benzodiazepines as needed and 5 (including 2 taking benzodiazepines) were prescribed antidepressants (3 sertraline, 2 bupropion). All subjects provided written, informed consent for institutional review board-approved study at the Mount Sinai School of Medicine.

Procedure

Subjects were instructed to collect urine samples over a 24-hr period, beginning after the first voided urine following awakening, and continuing through the first voided urine on the following day. Subjects were asked to collect urine on a day that was expected to be relatively quiet, and were provided collection containers to be kept on dry ice or in their freezer until brought to the laboratory. At this time they were questioned regarding completeness of the collection and about any potentially untoward events that may have occurred during the day of collection, occurrences which may have prompted a request for repeat collection. Cortisol and its metabolites were assayed in batched samples by electron impact GCMS, as described (Best & Walker, 1997). The limit of sensitivity for the glucocorticoid measures is 2ng injected on column. Specificity is less of an issue for GCMS than immunoassay, however no interferences were observed. Interassay coefficients of variations were <10% for cortisol and metabolites listed below. We also report data for urinary cortisol measured by radioimmunoassay (RIA) (Yehuda, 2002) to provide a basis of comparison with previous reports. RIA has been used extensively in biological psychiatry studies, and provides an estimate of total glucocorticoid output (including both active cortisol and its inactive metabolites, which are present due to cross-reactivity of the antigen).

Biologic variables of interest

Figure 1 provides a summary of the biosynthetic pathways related to cortisol metabolism measured in the current study. Total glucocorticoids was calculated as the sum of cortisol (F) and cortisone (E), and their metabolites, 5α-tetrahydrocortisol (5α-THF), 5β-THF and 5β-tetrahydrocortisone (5β-THE). 5α-reduction was inferred from the ratio of F/5α-THF, and 5β-

reductase activity from the ratio of F/5β-THF. Renal 11β-HSD-2 activity was inferred from the urinary free F/E ratio (Best & Walker, 1997).

Cardiometabolic disease manifestations

Non-insulin-dependent diabetes, hypertension, dyslipidemia and atherosclerotic heart disease were inferred on the basis of research chart review (by LMB) from the medical history in which all co-morbid medical diagnoses were recorded, list of current medications provided by the subject, responses on the medical questionnaire, and follow-up interview with the study physician. Note that this is an elderly population with ready access to health care so most routine cardiometabolic disease diagnoses had likely already been made. A positive notation for each diagnosis was recorded if it was provided in the medical history or if there was evidence of medical treatment or intervention for the condition (e.g., history of stent placement for atherosclerotic heart disease). Thus, subjects received a score between 0 and 4 derived by affording one point each for hypertension or treatment with antihypertensive medication; diabetes mellitus or treatment with oral hypoglycemic medication; dyslipidemia by history or report of medical management with anti-lipid agent and/or atherosclerotic heart disease inferred from history of myocardial infarction, angina, history of intervention or treatment for the latter; and body mass index (BMI) ≥ 26 kg/m^2.

Statistical Analysis

Group comparisons of survivors to controls were made with analyses of variance (ANOVA) and covariance (ANCOVA). Relationships among variables were assessed using partial correlation. Age, gender and BMI were used as covariates as noted. Bonferroni corrections were applied to analyses of covariance and to partial correlations. These corrections were applied separately for analyses involving glucocorticoids/metabolites and the enzyme indices.

RESULTS

Descriptive characteristics of Holocaust survivors and comparison subjects

There was a significantly greater proportion of males in the comparison (68%) than survivor (41%) group ($\chi^2_1 = 4.48$, p=.034). Survivors were significantly shorter in height, controlling for gender, but the groups did not differ in age, weight, BMI, or cardiometabolic manifestation scores. Thirty-one Holocaust survivors (61%) met criteria for lifetime PTSD, including 28 with current PTSD. Twenty survivors met criteria for lifetime major depression (39%), among whom 18 also had lifetime PTSD (90%; $\chi^2_1=11.78$, p=.001). Nine survivors met criteria for lifetime anxiety disorder other than PTSD, of whom 7 also suffered from lifetime PTSD (78%; $\chi^2_1=1.32$, *n.s.*). Only two subjects met criteria for current depressive disorder, and seven met criteria for current anxiety disorder at the time of study.

Although the comparison subjects were selected to be without lifetime history of PTSD or other psychiatric disorder, 10 of 22 reported having experienced traumatic exposures that were considered criterion-A events on review with a study clinician; in half these cases, the worst trauma occurred prior to the age of 15 (i.e., 'during development'). Three of the remaining five subjects described World War II military involvement. Though Holocaust survivors sometimes identified numerous traumatic exposures, when asked to identify their most salient trauma, subjects identified events that took place within months or a very few years of 12/31/1939.

Glucocorticoid measures in Holocaust survivors and controls

Samples for the survivors were collected between 04/29/1998 and 07/09/2003 and for the controls were collected between 09/05/1998 and 12/27/2003. Total 24-hr urinary free cortisol levels determined by RIA were significantly (26%) lower in Holocaust survivors (Table 1).

Similarly, the GCMS-derived total 24-hr glucocorticoid production rate, reflecting the sum of urinary F, E and their major metabolites, was significantly lower (39%) in survivors than controls. Interestingly, urinary free cortisol levels measured by GCMS did not differ between survivors and controls.

Analysis of urinary F, E and their individual metabolites by GCMS demonstrated that the overall reduction in glucocorticoids was primarily a result of a highly significant reduction (51%) in 5α-THF (Table 1). Accordingly, the ratio of F/5α-THF was increased, reflecting significantly lower 5α-reductase activity in Holocaust survivors. Holocaust survivors also showed modestly reduced urinary E, with a significantly elevated F/E ratio suggesting lower 11β-HSD-2 activity in the distal nephron, its major site of expression. In contrast, the other main pathway of glucocorticoid metabolism, 5β-reduction, was not significantly affected as evidenced by the similarity in survivors and controls in F/5β-THF, and correspondingly in levels of 5β-THF. There were no significant group differences in either urinary volume or creatinine concentrations that might have confounded the interpretation of the significant alterations observed, nor were there significant gender × group interactions for any measure. Repeating analyses without adjustment for age, gender and BMI, and/or using log-transformed values did not alter the significance of the findings. When the analyses for glucocorticoids were repeated in the Holocaust sample subdivided by PTSD diagnosis, there were no group differences (with p<.05) in glucocorticoids/metabolites or indices of enzyme function. Similarly, subdivision on the basis of lifetime major depressive disorder (MDD) yielded no significant subgroup differences.

Relationships between age at exposure and subsequent glucocorticoid metabolite and enzyme characteristics in Holocaust survivors

To examine whether any of the clinical or metabolic variables of interest might be related to critical periods of susceptibility to developmental events we examined correlations of approximate age of initial exposure to the Holocaust with glucocorticoid metabolites, cardiometabolic manifestation scores and PTSD variables, controlling for current age and gender. Total urinary glucocorticoids were positively correlated with age at exposure more than half a century earlier, with lowest glucocorticoid production rates in those youngest at exposure (Table 2). This association reflects the highly significant, positive correlation of 5α-THF output with age at exposure. Moreover, the significant negative correlation between age during the Holocaust and the ratio of F/5α-THF suggests lower 5α-reductase activity with younger age at exposure (Table 2). A significant negative correlation was also observed between age during the Holocaust and the ratio of urinary F/E, suggesting lower 11β-HSD-2 in adulthood among those who were younger at 'exposure'; the latter association, however, did not meet the Bonferroni criterion. 5β-reduction was not associated with age at the close of December, 1939.

Comparison subjects demonstrated similar positive associations of age at worst trauma exposure with total glucocorticoids and 5α-THF, but worst lifetime trauma exposure was not significantly associated with 5α-reductase or F/E.

Relationships with PTSD severity and trauma-related symptomatology

Although no differences in glucocorticoid metabolites or enzyme measures were noted between Holocaust survivors with and without PTSD, there was a significant association between 5α-THF and severity of PTSD using the continuous measure of CAPS lifetime total score (r=.479, n=50, p<.0005). This relationship was maintained after controlling for age of exposure, gender, and BMI (r=.462, df=45, p=.001), indicating that those with lower 5α-THF concentrations had lower lifetime PTSD symptom severity (Table 2). The relationship of lower 5α-THF with lower PTSD symptom severity was apparent within the subset of survivors with PTSD (simple r=.

510, n=30, p=.004, partial r=.544, df=25, p=.003). Other than an association of PTSD severity ratings with total glucocorticoids, which principally reflects the contribution of 5α-THF, no other metabolites were significantly correlated with PTSD severity, after applying Bonferroni corrections. Within the PTSD subgroup, however, the negative correlation between F/E and PTSD severity ratings was stronger (simple r= -.481, n=31, p=.006; partial r= -.477, df=26, p=.010), substantiating an association between diminished renal 11β-HSD-2 activity and lower PTSD symptom expression.

While CAPS scores were not obtained for the comparison subjects, Mississippi Scale scores were available for both groups. As was seen with the CAPS, survivors showed positive associations for Mississippi scale scores with both total glucocorticoids and 5α-THF levels whereas in the comparison subjects these associations were negative, and differed significantly between the groups (for total glucocorticoids, p<.0005; for 5α-TH, p=.0002). In the Holocaust survivor sample, there are no substantive relationships between Mississippi Scale scores and any other glucocorticoid, metabolite or enzyme. This is not the case for the controls, for whom negative correlations for 5β-THF (p=.067), THE (p=.063), and a positive correlation for 5β-reductase (p=.090) suggest an alternate pattern involving β-reduction.

Relationships with cardiometabolic manifestations scores

Correlations with the cardiometabolic manifestations scores showed only low cortisone and a significantly low F/E ratio, indicating higher scores associated with diminished renal 11β-HSD-2 (Table 2). Again, among survivors with PTSD, these relationships were more apparent: for E (simple r= -.424, n=31, p=.016; partial r= -.414, df=26, p=.029) and for F/E (simple r=. 595, n=31, p<.0005; partial r=.555, df=26, p=.002). Although the measure was composed in part of a nominal index of BMI elevation, covariation by a continuous measure of BMI did not alter the finding.

No significant difference in age ± SD of exposure was observed between those with (11.2±5.4 years) and without (12.6±7.5 years) PTSD. Age of exposure was also not significantly associated with cardiometabolic manifestations (r= -.106, df=46, *n.s.*). However, cardiometabolic manifestations scores were significantly reduced in survivors without PTSD (marginal x±SE: .56±.22) than in those who had developed the disorder (1.44±.18), covaried for age and gender (F=9.45, df=1,47, p=.004; Figure 2). This comparison retained significance when additionally covaried for BMI (F=5.59, df=1,46, p=.019). Cardiometabolic manifestations scores did not differ significantly for survivors with and without lifetime MDD, covaried for age and gender (marginal x±SE; with MDD (n=20) 1.39±.25; without MDD (n=31): .91±.20; F=2.17, df=1,47, *n.s.*).

These results again contrast to those found for the comparison sample: age at worst trauma (or age at the earliest trauma if more than one significant traumatization) was positively correlated with cardiometabolic manifestations score (partial r=.545 df=18, p=.013, covaried for current age and gender; partial r=.620, df=19, p=.003, covaried only for gender). For the controls, there was also a significant negative correlation between age at earliest trauma and Mississippi Scale score (r= -.689, df=18, p=.001, covaried for age, gender; r= -.614, df=19, p=.003, covaried only for gender).

DISCUSSION

We found that exposure to extreme psychological trauma or to prolonged physiological deprivation during development is associated with lowered glucocorticoid production rate and alterations in specific glucocorticoid metabolic pathways in old age. The most striking findings were (i) reduced total glucocorticoid production in Holocaust survivors, paralleling previous data showing lower cortisol levels in urine, saliva and blood in Holocaust survivors with PTSD,

but in this case similarly affecting survivors whatever their PTSD status (ii) This appeared largely due to lower excretion of the 5α-reduced cortisol metabolite 5α-THF. The higher F/5α-THF ratio implies lower levels of 5α-reductase. (iii) Glucocorticoids as well as glucocorticoid enzyme indices are associated with cardiometabolic disease manifestations among Holocaust survivors.

The Holocaust survivors in this study were several centimeters shorter than the comparison subjects, after gender and age correction, suggesting that prolonged nutritional deprivation impacted substantially on the developmental histories of a number, if not a majority, of the survivor sample. Although we assessed the impact of trauma related symptoms in survivors and comparison subjects, exposures for these groups differed vastly. Trauma for the survivors was not only substantially more severe, but also included prolonged nutritional deprivation. If the only difference was in intensity of trauma, one would anticipate associations in the same direction, but stronger for the Holocaust survivors. That the observed associations of 5aTHF with Mississippi scores were in the opposite direction for the survivors is therefore plausibly attributed to the effects of starvation. In addition, he mean age of trauma exposure among survivors is less than 15 years, and less than one half that of the comparison subjects; observed differences between these groups may equally be a consequence of the timing as of the type or severity of insult. Thus, an additional conclusion of this study is that reductions in 5α-reductase, and likely also 11β-HSD2, appear to be associated with starvation, or other trauma specific to the Holocaust survivors in this study, and may reflect their occurrence within a vulnerable developmental window.

5α-reductase is highly expressed in liver and male reproductive organs (Russel & Wilson, 1994). However, men and women exposed to the Holocaust showed similar lowering of 5α-reductase, suggesting a predominant effect upon hepatic 5α-reductase, presumably the type 1 isozyme. Long-term increases in 5α-reductase are associated with obesity and insulin-resistance (Rask et al, 2001; Andrew et al, 2002). Adult obesity is not a likely explanation for the findings in this study since survivors and controls were comparable in BMI, and the lowered levels of 5α-reductase in survivors persisted after adjustment for BMI (as well as age and gender).

Hepatic 5α-reductase is acutely down-regulated by calorie restriction, recovering on refeeding (Johnstone et al, 2004). Interestingly, in rodents, hepatic 5α-reductase is also sensitive to permanent 'programming' by perinatal events (Gustafsson & Stenberg, 1974). Exposure to the Holocaust in early life may have lowered hepatic 5α-reductase. The immediate consequence of lowered hepatic 5α-reductase would be a decrease in cortisol breakdown, potentially amplifying local glucocorticoid effects in the liver, and perhaps other metabolic tissues, without elevation of circulating cortisol and the consequent detrimental catabolic effects on muscle and CNS. This may have a survival advantage during starvation. That this 'expected' response during exposure appears to have persisted implies some form of 'programming'. This notion is supported by the relationship between estimated age at exposure and the degree of change in 5α-reduction in old age. Perhaps those who were youngest were exposed during a window of particular sensitivity to persisting effects. As suggested above, we interpret the decrement in height of Holocaust survivors as a likely reflection of extended nutritional deprivation and/or stress (which inhibits growth hormone and its signaling) during stages of development critical for growth. This might also have contributed to the persistence of changes in glucocorticoid metabolism among the survivors in this study since, at least in rodents, many of the key enzymes are regulated by growth hormone (Shimada et al, 1997). Lower 5α-reductase activity decreases total body cortisol turnover, lowering the 'basal' HPA axis activity required merely to maintain circulating cortisol levels and perhaps attenuating stress responses, consistent with some previous findings in Holocaust survivors (Yehuda, 2002; Yehuda et al, 2005b). Interestingly, lower 5α-THF in adulthood correlated with less severe PTSD symptoms.

This might reflect some metabolic protection from subsequent mental health risks although it is equally plausible that earlier exposure results *inter alia* in altered metabolism of cortisol and better psychological health without association between the two consequent parameters.

Holocaust survivors also had elevated urinary F/E ratio implying reduced activity of 11β-HSD-2 in the kidney. This effect was also associated with age at Holocaust exposure and PTSD symptom severity. Reduced renal 11β-HSD-2 allows illicit activation by cortisol of mineralocorticoid receptors in the distal nephron, increasing sodium retention (Walker et al, 1993), elevating plasma volume and blood pressure. Less cortisol metabolism by 11ß-HSD-2 also further reduces cortisol clearance, attenuating HPA axis drive (Lovati et al, 1999). Again, all these effects are expected to be of survival benefit during starvation/salt depletion, but their persistence may lead to later hypertensive consequences. Interestingly, in the current cohort hypertension trended more common in survivors with PTSD (14/31) than in those without PTSD (4/20; χ^2_1=3.37, p=.066). Unfortunately, direct measures of unmedicated blood pressure could not be obtained, but prescription of medication and history of hypertension seem a fair proxy in these subjects who have good access to health care. Hypertension has been linked with combat trauma in Vietnam veterans (Eberly & Engdahl, 1991), and has been associated with childhood physical and sexual abuse (Davidson et al, 1991), as well as prenatal glucocorticoid exposure (Doyle et al, 2000).

Indeed, although all survivors were directly exposed to the Holocaust, prenatal events might also be relevant. In light of the role of placental 11β-HSD-2 in glucocorticoid programming of the fetus and subsequent adult HPA axis and metabolic functions (Benediktsson et al, 1993; Lindsay et al, 1996), there might be at least some contribution from effects of extreme stress on the pregnant mothers of these subjects. That is, as many Jews came to feel increasingly unsafe in the years leading to World War II, it is plausible that some infants born in close proximity to the Holocaust were exposed to higher levels of maternal glucocorticoids.

Though the relative contributions of maternal influences and direct effects of the survivor's experience cannot be distinguished, the findings demonstrate that Holocaust survivors who were younger at the time of exposure to the Holocaust were better able to maintain a larger portion of bioactive cortisol by decreasing the rate of cortisol metabolism. Under such conditions, the effects of glucocorticoids within specific cell types could be amplified despite a reduced rate of adrenal glucocorticoid production, which in turn could contribute to changes in glucocorticoid receptor regulation of cortisol (Lovati et al, 1999; Funder et al, 1988). Enhanced tissue responsiveness to glucocorticoids has been demonstrated in PTSD, with those younger at age of trauma having the greatest enhancement as reflected by a lower the lysozyme $IC_{50\text{-DEX}}$ [nM] of cultured mononuclear leukocytes (Yehuda et al, 2004).

In the current study, no differences were observed in cortisol levels, metabolites or enzyme indices in Holocaust survivors based on presence or absence of PTSD, even when RIA was used. This may reflect that with advancing age, the distinction between survivors with and without PTSD becomes less marked, though as a group, survivors become further differentiated from non-exposed subjects, as recently demonstrated by longitudinal studies (Yehuda et al, 2006; Yehuda et al, 2007). In contrast, cardiometabolic disease manifestation scores were higher in Holocaust survivors with PTSD, whereas survivors who did not develop PTSD appeared to resist cardiometabolic diagnoses. Thus, the development cardiovascular risk factors as well as the development of stress related disorders may represent the product of a trajectory set in motion in early life rather than a correlate of more contemporary determinants of mental health symptom severity and/or trauma-related pathophysiology.

This study has clear limitations including (i) reliance on retrospective chart review and medical history for the assignment of a cardiometabolic manifestations score; (ii) inferring maternal/

early childhood nutritional status; (iii) imperfect gender matching, although no gender differences were noted for any metabolite or enzyme in either the survivor or comparison samples; and (iv) a lack of data pertaining to the post-war care environment, which may moderate late life physical and emotional well-being, particularly for the youngest child Holocaust survivors (van der Hal-van Raalte et al., 2007; 2008).

In sum, the differences observed between Holocaust survivors and comparison subjects in two important glucocorticoid-related metabolic enzyme pathways provides a rationale for examination of these parameters in other groups of trauma survivors. Future studies should further examine tissue-specific alterations in the metabolism of glucocorticoids in other cohorts to determine the extent to which these serve as compensatory mechanisms to mitigate the detrimental neuroendocrine and metabolic effects of stress in early life.

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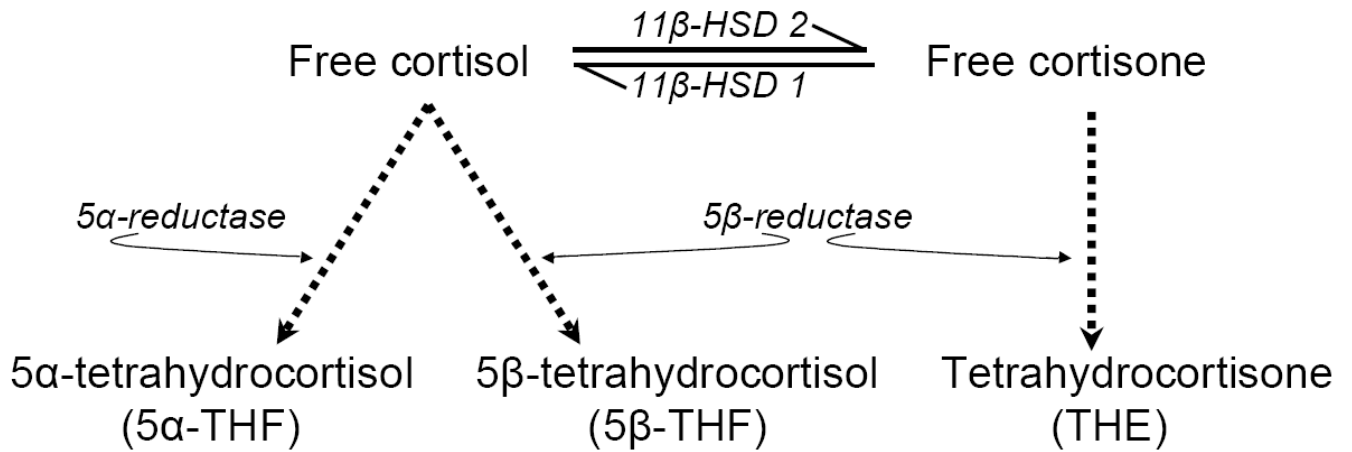
Figure 1. Summary of Metabolic Pathway
11β-HSD is 11β-hydroxysteroid dehydrogenase. 11ß-HSD type 1 (mainly located in liver, adipose tissue and brain) catalyses the reduction of inert cortisone to active cortisol; 11ß-HSD type 2 oxidizes active cortisol to inert cortisone in the kidney. 5α- and 5β -reductase are the rate limiting enzymes in the conversions of cortisol to 5α-tetrahydrocortisol (THF) and 5β-THF, respectively, largely occurring in liver, but also the brain. 5β-reductase is also rate limiting in the conversion of cortisone to tetrahydrocortisone (THE).

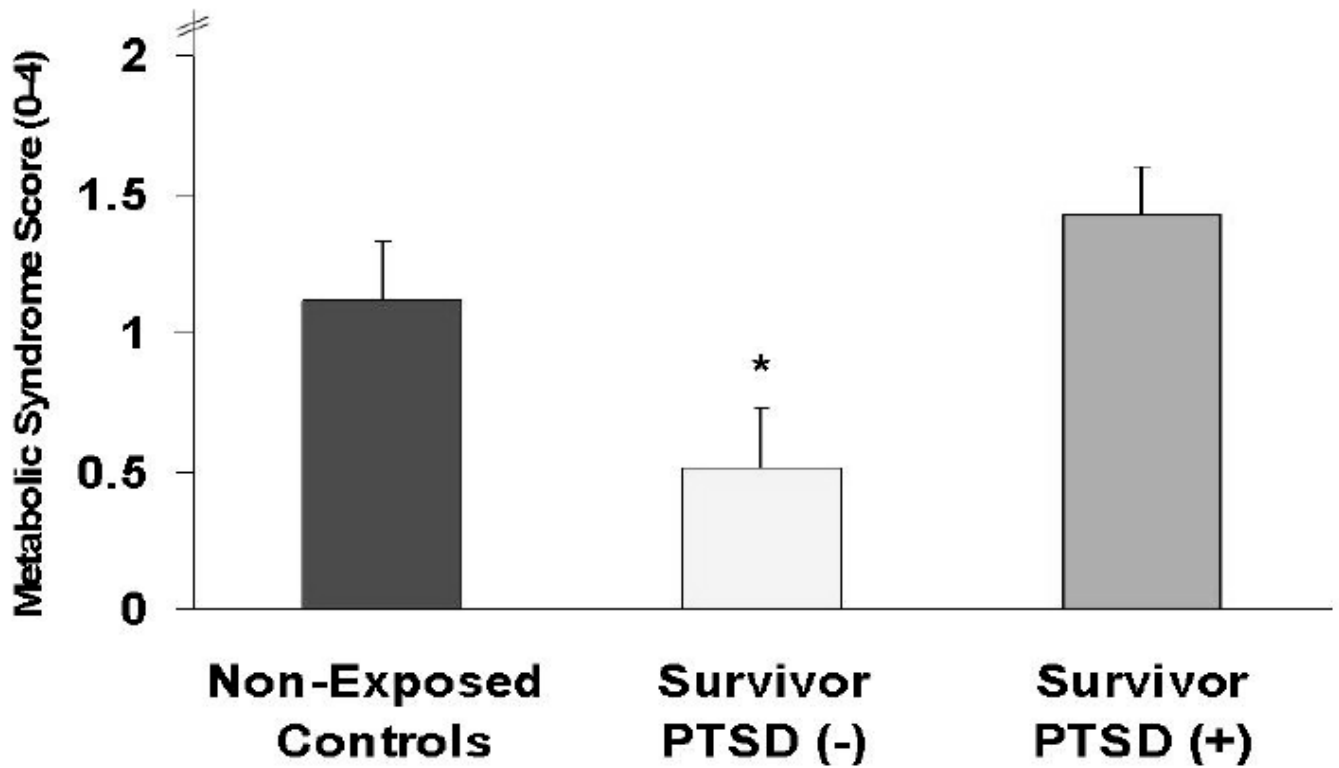**Figure 2. Cardiometabolic manifestation scores in Holocaust survivors with and without PTSD and in non-exposed subjects**
The three groups differ significantly (F=5.40, df=2,68, p=.007), controlling for age (p=.005) and gender (*ns*), with post-hoc tests (LSD) demonstrating scores for survivors without PTSD to be significantly lower than those for survivors with PTSD (p=.002) and comparison subjects (p=.053).

Table 1

Comparison of group characteristics, metabolic variables, and glucocorticoid measures between Holocaust survivors and comparison subjects.

	Comparison subjects (n=22)	Holocaust survivors (n=51)	ANCOVA[2]
	mean ± SE[1]	mean ± SE	F (df) p
I. Group characteristics			
Age (years)	71.1 ± 1.6	73.1 ± 1.0	1.06 (1,71) ns
Age, December 31, 1939	11.6 ± 6.8	11.8 ± 6.3	.006 (1,71) ns
Age, trauma exposure (earliest specified)	34.6 ± 22.2	14.4 ± 7.6[3]	33.7 (1,71) <.0005
Height (cm)	170.4 ± 1.5	163.8 ± 1.0	14.95 (1,70) <.0005
Weight (kg)	73.0 ± 2.5	70.7 ± 1.7	.54 ± (1,70) ns
BMI (kb/m^{2})	25.0 ± .8	26.3 ± .6	1.63 (1,71) ns
Cardiometabolic manifestations score[4]	1.11 ± .23	1.07 ±.15	.02 (1,69) ns
Mississippi Scale score	63.4 ± 12.2	98.4 ± 24.8	39.4 (1,71) <.0005
Urine volume	1790.3 ± 166	1471 ± 106.9	2.49 (1,68) ns
RIA - Cortisol (μg/day)	51.2 ± 3.8	37.6 ± 2.4	8.75 (1,67) .004
II. Glucocorticoids /metabolites[5]			
Total glucocorticoids [GCMS][6]	12086 ± 1060	6514 ± 587	**9.15 (1,55) .004[7]**
- F (cortisol)	100.6 ± 9.7	100.2 ± 6.3	.00 (1,68) ns
- E (cortisone)	142.7 ± 11.6	106.6±7.5	6.52 (1,68) .013
- α-THF (5α-tetrahydrocortisol)	5157 ± 616	2528 ± 318	**12.64 (1,65) .001**
- β-THF (5β-tetrahydrocortisol)	1952 ± 362	1561 ± 215	.81 (1,57) ns
- THE (tetrahydrocortisone)	2848 ± 364	1926 ±220	4.46 (1,63) .039
III. Enzyme indices[8]			
- F/ α-THF (5α-reductase)	.035 ± .023	.106 ± .014	**6.57 (1,65) .013**
- F/ β-THF (5β-reductase)	.073 ± .009	.080 ± .005	.47 (1,57) ns
- F/E (11β-hydroxysteroid dehydrogenase 2)	.777 ± .077	1.045 ± .049	**.831 (1,68) .005**

[1] Adjusted mean ± standard error of the mean, ns: p>.05

[2] ANCOVA, for height, weight, controlling for gender; for cardiometabolic manifestations score, controlling for age, gender; for urine volume, and all glucocorticoid variables, controlling for age, gender, BMI

[3] The discrepancy between this age and age as of December 31, 1939 is primarily accounted for by one Survivor who was only 3.5 years old at the end of World War II, and was gang raped at age 41.

[4] Described in text; equal to the sum of one point each for atherosclerotic heart disease and/or dyslipidemia, hypertension, diabetes, BMI ≥ 26.

[5] All values represent urinary steroids in μg/24hr.

[6] Total glucocorticoids = F + E + α-THF + β-THF + THE.

[7] Bold face indicates significance by Bonferroni criterion (6 comparisons for glucocorticoids/metabolites; 3 comparisons for enzyme indices).

[8] Increased values for listed enzyme indices indicate reduced activities.

Table 2

Relationships among glucocorticoid measures, age at the end of 1939, trauma-related symptoms and cardiometabolic scores among Holocaust survivors and comparison subjects

	Comparison subjects (n=22)				Holocaust survivors (n=51)				
	n	Age earliest trauma[1] r,p[6]	Trauma-related symptoms[2] r,p	Cardio-metabolic score[3] r,p	n	Age initial exposure[4] r,p	Trauma-related symptoms r,p	PTSD Severity[5] r,p	Cardio-metabolic score r,p
I. Glucocorticoids and metabolites:									
Total glucocorticoids[7]	15	.658,.015	**-.770,.001**	.455, ns	45	**.438,.003**	.328,.011	**.423,.005**	.050, ns
Cortisol (F)	22	-.153, ns	.166, ns	.231, ns	51	-.227, ns	.040, ns	.098, ns	-.018, ns
Cortisone (E)	22	-.321, ns	.269, ns	.170, ns	51	.079, ns	.080, ns	.253, ns	-.321,.025
5α-THF[8]	20	.543,.020	-.481,.043	.299, ns	50	**.489, <.0005**	**.480,.001**	**.480,.001**	.019, ns
5β-THF[8]	15	.347, ns	-.522, ns	.352, ns	45	.061, ns	.078, ns	-.010, ns	.018, ns
THE[7]	19	.279, ns	-.460, ns	.298, ns	49	-.126, ns	.019, ns	.228, ns	.278, ns
II. Glucocorticoid enzyme indices:									
5α-reductase (F /α-THF)	20	-.007, ns	.116, ns	.321, ns	50	**-.373,.009**	-.107, ns	-.256, ns	-.078, ns
5β-reductase (F /β-THF)	15	-.273, ns	.488, ns	-.145, ns	45	-.267, ns	-.175, ns	-.153, ns	-.289, ns
11β-HSD-2 (F/E)[9]	22	.138, ns	-.165, ns	.015, ns	51	-.319,.026	-.150, ns	-.298,.038	**.351,.014**

[1] Age at earliest trauma for comparison subjects was 34.6 ± 22.2 years, range 6-81.

[2] Trauma-related symptom severity determined as Mississippi Scale score.

[3] Cardiometabolic manifestations score equal to the sum of one point each for atherosclerotic heart disease and/or hyperlipidemia, hypertension, non-insulin dependent diabetes mellitus, and BMI $\geq 26 kg/m^2$.

[4] Operationalized as age as of December 31, 1939; for survivors this age (mean ± SD) was 11.8 ± 6.3 years, range 0-27 years.

[5] PTSD severity determined using the Clinician Administered PTSD Scale - Lifetime total score.

[6] Partial correlations; all correlations covaried for current age and gender.

[7] Total glucocorticoids = F + E + 5α-THF + 5β-THF + THE.

[8] 5α-THF: 5-alpha-tetrahydrocortisol; 5β-THF: 5-beta-tetrahydrocortisol

[9] THE: tetrahydrocortisone

[10] 11β-hydroxysteroid dehydrogenase type 2

[11] Bold face indicates significance by Bonferroni criterion for glucocorticoids/metabolites (6 comparisons); for enzyme indices (3 comparisons).