The Natural History of Neuroendocrine Changes in Pediatric Posttraumatic Stress Disorder (PTSD) After Motor Vehicle Accidents: Progressive Divergence of Noradrenaline and Cortisol Concentrations Over Time

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Background: The hypothalamic-pituitary-adrenal axis and the catecholaminergic system are involved in the pathophysiology of posttraumatic stress disorder (PTSD). This was a prospective and longitudinal study of neuroendocrine physiology in children with PTSD following a motor vehicle accident (MVA).

Methods: Sixty children aged 7–18 were studied immediately after an MVA and 1 and 6 months later. Fasting morning plasma catecholamine and serum cortisol concentrations were measured. Salivary cortisol concentrations were measured serially five times daily to examine circadian variation in all three assessments. Values were compared between those who did (PTSD) or did not develop PTSD (non-PTSD) after the trauma and a control group at months 1 and 6.

Results: Twenty-three of the children had PTSD at the 1-month and 9 children at the 6-month evaluations. 1) Plasma noradrenaline concentrations were higher in the PTSD group than in the other two groups at both months 1 and 6 ($p = .001$ and $p = .001$, respectively). Additionally, the PTSD patients presented with significantly higher salivary cortisol concentrations at 18.00 ($p = .03$) and 21.00 ($p = .04$) at month 1.2) Eight children suffering from PTSD at both months 1 and 6 had significantly elevated plasma noradrenaline concentrations at month 6 compared with those at month 1 and at baseline and to the other two groups (within subjects: $p < .001$; between subjects: $p = .005$). The initially elevated evening salivary cortisol concentrations in this group normalized at month 6.

Conclusions: This progressive divergence of noradrenaline and cortisol concentrations over time might underlie the natural history and pathophysiology of PTSD.

Key Words: Childhood, development, motor vehicle accidents, plasma catecholamines, PTSD, salivary cortisol

Posttraumatic stress disorder (PTSD) develops after exposure to events that involve a threat to the physical integrity of the individual or others and evokes intense fear, helplessness, or horror. Symptoms include reexperience of the initial trauma, avoidance of stimuli associated with the trauma, and symptoms of excessive arousal. By definition, PTSD lasts over 4 weeks (1). Motor vehicle accidents (MVAs) are major stressors and causes of PTSD. Thirty to 80% of children may exhibit full PTSD symptomatology after MVAs (2,3). In adults with PTSD, 24-hour urinary catecholamine excretion and/or plasma catecholamine concentrations are higher than those of control subjects (4–9). In the majority of studies, these patients have elevated basal cerebrospinal fluid (CSF) corticotropin-releasing hormone (CRH) concentrations (10), while variable findings have been reported regarding cortisol concentrations in the periphery (11): low or normal 24-hour urinary excretion of free cortisol and low or normal plasma and salivary cortisol concentrations (11–17). In cases of PTSD and comorbid major depression, high urinary cortisol concentrations have been reported (18).

Children with PTSD have increased urinary catecholamine excretion. However, unlike adults, their plasma, salivary, and urinary cortisol concentrations are usually normal or high. A recent report (19) on PTSD development after the impact of an acute stressor such as an MVA showed increased initial cortisol secretion after the trauma, and a second report (20) of pediatric PTSD shortly after the trauma showed high salivary cortisol concentrations. In contrast to these findings, adolescents with PTSD 5 years after the trauma demonstrated low cortisol levels after the dexamethasone suppression test (21). Finally, variable cortisol levels were reported in maltreated children with or without a clinical diagnosis (22,23).

These findings raise two hypotheses: first, acute stressors and chronic repeated trauma, such as child maltreatment, may alter neuroendocrine mechanisms differentially, resulting in diverse cortisol secretory patterns. Second, low cortisol, a frequent finding in adult studies, may either reflect previous psychopathology, and thus vulnerability to PTSD, when found immediately after the accident or it may be the neuroendocrine end stage of the natural history of the disorder, months or years after the trauma.

We hypothesized that a healthy young population, screened for psychopathology and concurrent use of alcohol or drugs, after the impact of a single acute stressor such as a car accident may be an ideal population for an observational study regarding the longitudinal neuroendocrine profile of the disorder. We investigated the changes in the two major neuroendocrine axes

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in relation to each other and in relation to psychopathology in the children that went ahead to develop PTSD. Our hypothesis was that trauma might initially increase cortisol concentrations and disturb cortisol rhythm in this healthy young population. Cortisol secretion might gradually normalize or become low, whereas maintenance or further elevation of high noradrenaline concentrations might result in persistence of PTSD clinical symptomatology through time.

The purpose of this study was to investigate the behavioral and neuroendocrine pathophysiology of PTSD in childhood and adolescence in a prospective and longitudinal manner. To accomplish this, we determined the relation between full PTSD diagnosis or specific clusters of posttraumatic symptoms and stress hormone concentrations immediately and 1 and 6 months after a MVA.

Methods and Materials

Participants

Ethical approval for the study was obtained from the Aghia Sophia Children's Hospital Ethics Committee. The study was conducted from May 2002 to December 2004, over a 2.5-year period. Children and adolescents aged 7–18 years were seen serially after an MVA. Informed written consent was obtained from the participants and their parents.

Sixty children and adolescents who had experienced an MVA, were hospitalized for at least 1 day, and met the criteria of the study were included (Table 1). Inclusion criteria were 1) experienced an MVA as a pedestrian or a passenger, defined as a traumatic event by DSM-IV criterion A; 2) were clinically evaluated in the first 24 hours after the accident, and the degree of physical injury was assessed at the surgical, neurosurgical, and/or orthopaedic out-patient clinics; 3) were hospitalized for at least 1 day; and 4) the family was willing to participate in the project. Exclusion criteria were 1) major brain injuries (Glascow Coma Scale [GCS] $<$ 13); 2) underlying chronic illnesses and chronic use of medication, including steroid contraception; 3) mental illnesses or preexisting psychiatric disorders; 3) learning difficulties; 4) inability to speak, read, and write in Greek; 5) corticosteroid treatment during hospitalization; and 6) family history of serious chronic illnesses including mental or psychiatric disorders.

The study design included the following three assessments: a baseline evaluation shortly after the accident and two follow-up assessments 1 and 6 months after the accident. Of the initial population, 56 children (40 boys and 16 girls) and their parents completed the first two assessments, and 47 (32 boys and 15 girls) completed all three. Four subjects could not be located for the second evaluation, and the rest elected to discontinue the study. The participants who dropped out did not differ from those who completed the study regarding to age, body mass index (BMI), and sex ratio. Figure 1 depicts patient participation in the study.

Table 1. Patient Clinical Profile (Mean ± SD)

	Total Sample	Boys	Girls
Patients	(n = 60)	(n = 40)	(n = 20)
Age	10.70 ± 2.46	10.66 ± 2.71	10.79 ± 1.95
Race (White)	60	40	20
Prepubertal	32	22	10
Pubertal	28	18	10
BMI-SDS	.38 ± 1.31	.29 ± 1.31	.63 ± 1.29
Control Subjects	(n = 40)	(n = 13)	(n = 27)
Age	10.49 ± 2.59	10.90 ± 2.50	10.30 ± 2.66
Race (White)	40	13	27
Prepubertal	26	8	18
Pubertal	14	5	9
BMI-SDS	.37 ± 1.63	.11 ± 1.69	.50 ± 1.62

BMI-SDS, body mass index in standard deviation scores.

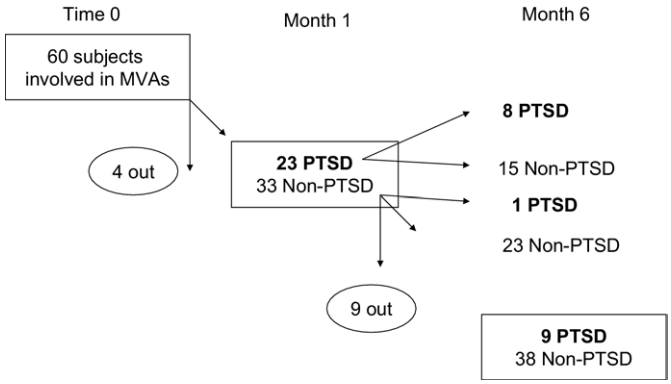

Figure 1. Participants and group formation longitudinally.

A healthy control group (n = 40) of the same age and BMI participated in the study (Table 1). This sample was derived from the healthy, normal BMI siblings of children followed at the obesity clinic of our department. The children with simple obesity had no underlying endocrine or genetic disorders. First-degree relatives of the control group had no history of serious chronic physical or mental illness.

Clinical Evaluation

Sociodemographic details and past medical and psychiatric history were recorded. Children's BMI was calculated from the weight in kilograms divided by the square of the height in meters. BMI standard deviations were calculated based on Greek growth charts (24).

Pubertal Assessment. The pubertal development of the participants was determined by physical examination by a certified paediatrician based on the Tanner staging system of pubic hair and genital development in boys and pubic hair and breast development in girls (25).

To provide an overall severity score for patients with multiple injuries, the Injury Severity Score (ISS) was computed using objective injury data collected from patient charts. The ISS was classified as ISS 1–3, ISS 4–8, and ISS ≥ 9 to indicate minor, moderate, and serious severity, respectively.

Diagnostic Instruments

Children and their parents were invited to participate in the study and were given baseline questionnaires, covering details of the incident and the educational level of the parents as an indicator of their socioeconomic status.

1. Diagnostic Interview. The children were interviewed with the PTSD part of the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS)—Lifetime version (K-SADS-PL) interview for school-age children. This is a semistructured diagnostic interview for children and adolescents aged 6–17 years and their parents that assesses the frequency and intensity of the three DSM diagnostic criteria for PTSD. A single, board-certified child psychiatrist who was trained on the administration of this instrument conducted the interview. All experimental subjects were interviewed with the K-SADS-PL and K-SADS instruments at

the 1- and 6-month assessments. The K-SADS-PL version used in this study gave DSM-IV psychiatric diagnoses (26).

2. Self-Completed Questionnaires. All participants and their parents were asked to complete the following self-report questionnaires:

- The Youth Self Report (YSR, 27) and the Children's Behavior Checklist. (CBCL, 28) (Using both these diagnostic instruments, individuals were assessed for previous psychopathology.)
- The Children's Posttraumatic Reaction Index (CPTS-RI, 29) and the adult version for parents.
- The Posttraumatic Stress Diagnostic Scale (PDS, 30) for parents.
- The State–Trait Anxiety Inventory—Children (STAIC, 31).
- The Impact of Event Scale (IES, 32), a measure of subjective stress for adults involved in the accident.
- The Life Events Scale, a diagnostic instrument that assesses previous trauma and is completed by the parents (33).

Study Groups

Based on the PTSD part of K-SADS interview, patients were divided into two groups, PTSD and non-PTSD, evaluated 1 and 6 months postaccident (Table 2). Cross-sectional evaluation examined differences between PTSD and non-PTSD participants separately at each time point. Longitudinal evaluation included the continuous PTSD and non-PTSD groups for months 1 and 6, where those exhibiting both diagnoses over time were not included in the repeated-measures analysis.

Neuroendocrine Evaluation

Salivary cortisol was obtained from participants during hospitalization. Salivary samples were collected five times a day (8 AM before breakfast, 12 NOON, 3 PM, 6 PM, and 9 PM). Detailed instructions were given to parents and children to collect the saliva samples properly using a Salivette device (Sarstedt, Nuembrecht, Germany). Participants were not receiving any medications and did not smoke. Children were told not to eat or exercise for at least half an hour before sample collection. Each of the samples was collected by having the participant place a cotton swab in his or her mouth for 2 min or chew it for 1 min. The cotton was then placed inside a plastic tube and kept in the refrigerator at 0–4°C. One day after collection, the samples were given to the investigator for further processing. Salivary cortisol was extracted from the cotton by centrifuging the plastic tubes and cotton at 1000 *g* for 8 min to separate the saliva into the outer tube. The cotton was then removed, and all samples were stored at −85°C. Samples were processed using the Elecsys Cortisol reagent kit (Roche, Basel, Switzerland).

Table 2. Posttraumatic Stress Disorder (PTSD) Group Characteristics (M ± SD)

Group	Age (years)	BMI-SDS	Sex
Month 1			
PTSD ($n = 23$)	11.25 ± 3.07	.85 ± 2.74	19 M, 4 F
Non-PTSD ($n = 33$)	10.76 ± 1.94	.55 ± 1.35	21 M, 12 F
Control ($n = 40$)	10.49 ± 2.59	.37 ± 1.63	13 M, 27 F
Month 6			
PTSD ($n = 9$)	11.53 ± 2.98	1.01 ± 1.2	8 M, 1 F
Non-PTSD ($n = 38$)	11.50 ± 2.46	.63 ± 1.64	24 M, 14 F
Longitudinal PTSD at Months 1 and 6			
PTSD ($n = 8$)	10.88 ± 3.36	.45 ± 1.01	7 M, 1 F
Non-PTSD ($n = 23$)	11.15 ± 2.01	.43 ± 1.38	15 M, 8 F

BMI-SDS, body mass index in standard deviation scores; F, female; M, male.

Serum cortisol concentration was measured in a fasting morning sample. Both serum and saliva were stored at −85°C. Cortisol was measured using an electrochemiluminescence immunoassay (Roche Co., Basel, Switzerland). The intra-and interassay precision coefficients of variation for serum cortisol were 1.1–1.3 and < 8% and for salivary cortisol ranged from 1.5 to 6.1 and 4.1% to 8%, respectively. The analytical sensitivity (lower detection limit) was < .036 μg/dL.

From the same blood sample, plasma was obtained for the measurement of catecholamines. Because venipuncture stress elevates catecholamine levels, samples were collected with the patient supine for 10–15 min after cannulation. Blood was collected in heparin tubes and immediately immersed in an ice bath. Samples were stored at −85°C, and catecholamine concentrations were measured using high performance liquid chromatography with electrochemical detection (Chromsystems Diagnostics, Munich, Germany). The intra- and interassay precision coefficients of variation for plasma catecholamines ranged from 1.7% to 11.4% and 3.7% to 12.7%, respectively. Serum, plasma, and saliva measurements were performed simultaneously at all three assessments.

Regarding the order of the diagnostic procedures, five salivary samples were collected during the day before blood sampling. In the follow-up assessments, salivary samples were collected at home, preferably on a Sunday, and the samples were given back to the investigator the next morning. At this time, a physical examination was performed, followed by blood sampling and a clinical interview. Questionnaires were completed at home during the weekend.

Regarding sampling in relation to medical procedures, the use of analgesics or antibiotics was not an exclusion criterion. Depending on the medical condition and the days of hospitalization, the usual process was as follows: every morning, the surgical departments of our hospital were screened for new admissions. Families were informed about the project, and questionnaires were given to those who gave written consent. When the samples and the questionnaires were returned, a physical examination was performed, and a fasting blood sample was obtained. Diagnostic interview was not performed during hospitalization (by definition, PTSD diagnosis occurs at least 1 month after the trauma).

Statistical Methods

SPSS was used for all data analyses, including descriptive statistics. To test the main hypothesis, a one-way analysis of variance (ANOVA) was conducted with all measurements as dependent variables and group (PTSD, non-PTSD, and control) as the between-subjects independent variable. For salivary cortisol, time of the day was tested as the within-subjects independent variable between the three groups.

Examining serial hormonal concentrations in the longitudinal PTSD (1 and 6 months) group, time (months after the accident) was tested as the within-subjects independent variable between the three groups. For multiple comparisons, a 3 × 3 mixed repeated-measures factorial analysis of variance (ANOVA) with one within-subjects factor—*time* (0, 1, and 6 months)—and one between-subjects factor—*group* (PTSD, non-PTSD, control)—was conducted to evaluate the effect of time and group. Because the sphericity assumption was violated ($p < .001$), the results of the tests of the within-subject effects were also evaluated with the Huynh–Feldt test. Adjustments were

made using Bonferroni corrections. The repeated-measures ANOVA included a non-PTSD subject with some missing information. The analysis was also done without this subject, and the results were similar.

Results

Relations Between PTSD Diagnosis or Posttraumatic Symptoms and Hormone Concentrations at Months 1 and 6

Twenty-three children (19 boys and 4 girls) had a PTSD diagnosis at the 1-month assessment, and 9 children (8 boys and 1 girl) had PTSD at month 6. In our study, there was a much higher percentage of boys involved in road accidents (40 boys and 20 girls) and a higher percentage of boys with PTSD.

Month 1. ANOVA revealed increased plasma noradrenaline levels in the PTSD population compared with the non-PTSD group and to normal volunteers [$F(2,81) = 8.142$, $p = .001$; Figure 2A]. Additionally, the PTSD group demonstrated elevated evening (6 PM) [$F(2,86) = 3.602$, $p = .03$] and night (9 PM) [$F(2,86) = 3.468$, $p = .036$] salivary cortisol concentrations compared with the other two groups (Figure 2B).

Examining separately the three symptom clusters, *arousal* symptoms at month 1 were related to significantly higher plasma noradrenaline levels compared with the other two groups [$F(2,81) = 3.320$, $p = .04$].

Reexperience symptoms were related to significantly higher afternoon (3 PM) salivary cortisol concentrations compared with the non-reexperience and control groups [$F(2,86) = 3.829$, $p = .03$].

Month 6. Patients who met full PTSD criteria at month 6 demonstrated higher plasma noradrenaline levels than those who did not and than normal control subjects [$F(2,72) = 7.514$, $p = .001$; Figure 2A].

For symptom dimensions and hormones, ANOVA revealed increased plasma noradrenaline concentrations in every symptom cluster examined separately: reexperience [$F(2,72) = 7.890$, $p = .001$], avoidance [$F(2,72) = 7.397$, $p = .001$], and arousal [$F(2,72) = 13.555$, $p < .001$]. Additionally, cortisol was significantly higher in morning (8 AM) serum [$F(2,50) = 4.107$, $p = .02$] and afternoon (3 PM) [$F(2,76) = 4.719$, $p = .012$; Tukey's: reexperience vs. non-reexperience, $p = .01$] and evening (9 PM) [$F(2,75) = 3.486$, $p = .036$] saliva in patients with reexperience symptoms. The group with hyperarousal symptoms also demonstrated significantly higher evening salivary cortisol (9 PM) than the other two groups [$F(2,75) = 3.857$, $p = .03$].

Impact of Injury Severity. Major injuries were excluded from the study. Trauma severity was assessed using the ISS, and there was no correlation between ISS and serum cortisol, salivary cortisol (morning and evening), or catecholamine concentrations immediately after the accident. To assess physical trauma severity in the groups studied, ISS means were compared between the PTSD and the non-PTSD group separately for months 1 and 6. *t* tests were performed between the PTSD ($n = 23$) and the non-PTSD ($n = 33$) groups. At month 1, ISS mean was 3.25 and 3.65, respectively [$F(56) = .961$, $p = .591$]. At month 6, ISS mean was 3.48 for the PTSD group ($n = 9$) and 5.2 for the non-PTSD group (n = 38) [$F(46) = 2.716$, $p = .205$].

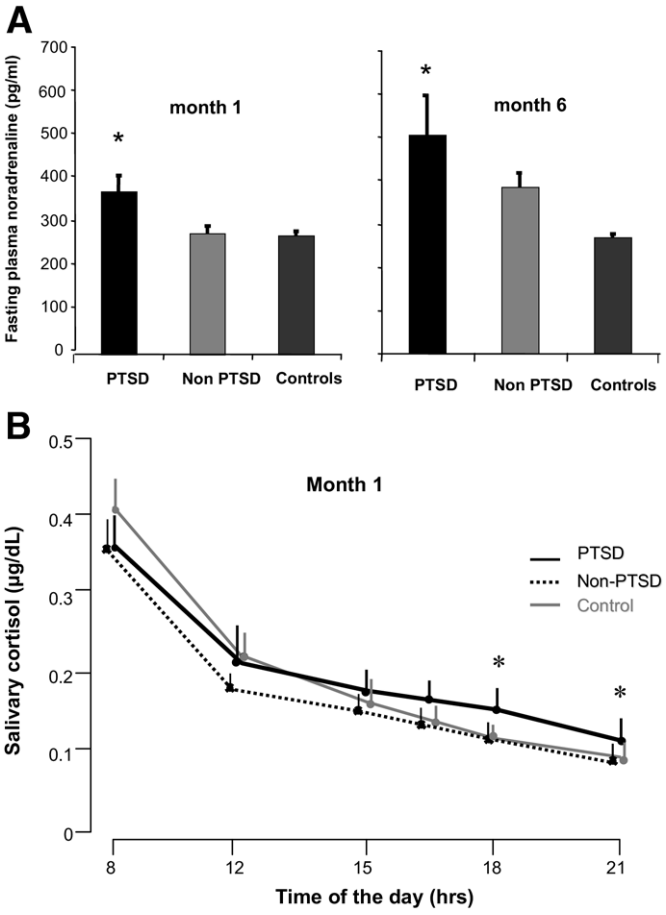

Figure 2. (A) Plasma noradrenaline concentrations (mean values and standard errors) at 1 and 6 months following motor vehicle accident. Posttraumatic stress disorder (PTSD) groups were defined cross-sectionally at each time point. Age- and body mass index (BMI)-matched control values are included (*p* value for both months 1 and 6, $p = .001$, Tukey's multiple comparisons for month 1: PTSD vs. non-PTSD: $p = .001$, non-PTSD vs. control subjects: $p = .9$, PTSD vs. control subjects: $p = .005$. For month 6: PTSD vs. non-PTSD: $p = .16$, Non-PTSD vs. control subjects: $p = .02$, PTSD vs. control subjects: $p = .002$). (B) Circadian salivary cortisol rhythm (mean values and standard errors) in PTSD and non-PTSD patients at month 1 after motor vehicle accident. Age- and BMI- matched control values are included. (Analysis of variance *p* value for 6 PM: .03; for 9 PM: .04. Tukey's multiple comparisons for 6 PM: PTSD vs. non-PTSD: $p = .03$, non-PTSD vs. control subjects: $p = .9$, PTSD vs. control subjects: $p = .09$. For 9 PM: PTSD vs. non-PTSD: $p = .03$, non-PTSD vs. control subjects: $p = .2$, PTSD vs. control subjects: $p = .6$).

Natural History of the Disorder

Of the 23 children suffering from PTSD at the month 1 assessment, 8 preserved full PTSD diagnosis at month 6. This group (PTSD 1 and 6 months) was compared with the non-PTSD (1 and 6 months) group and to normal controls. One patient with late-onset PTSD and the 15 diagnosed with PTSD only at month 1 and not at month 6 were excluded. Plasma noradrenaline was significantly elevated in the PTSD group compared with the other two groups at months 1 and 6. Furthermore, examining both PTSD and non-PTSD groups, noradrenaline concentrations at month 6 were higher than those at month 1 and at baseline (within-subjects effect: $p < .001$, between-subjects effect: $p = .005$). Adrenaline concentrations, like noradrenaline, were higher in both PTSD and non-PTSD groups at month 6 compared with those at month 1 and at baseline, but these differences were not statistically significant.

Interestingly, the non-PTSD group presented with a similar, albeit smaller, elevation in noradrenaline levels at month 6. However, it should be noted that the non-PTSD group was, in fact, a group of children with partial PTSD, given that PTSD

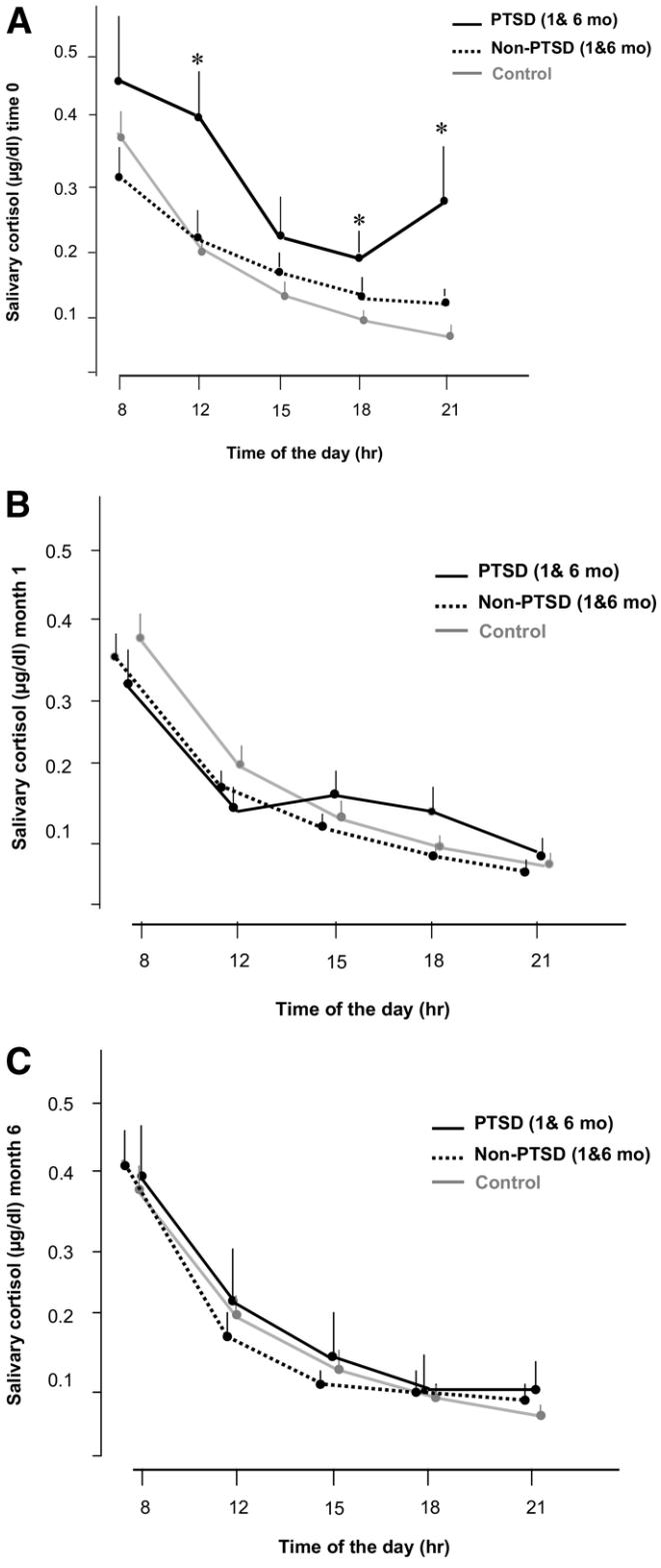

Figure 3. Circadian salivary cortisol rhythm immediately and 1 and 6 months after motor vehicle accident in the longitudinal posttraumatic stress disorder (PTSD) group (subjects who had the diagnosis at month 1 and maintained it though month 6, $n = 8$), non-PTSD (subjects without PTSD at both months 1 and 6, $n = 23$), and control subjects. (A) Analysis of variance p value for 12 noon = .006; for 6 PM = .014; for 9 PM <.001. Figures 3 (B) and (C): No significant differences were noted.

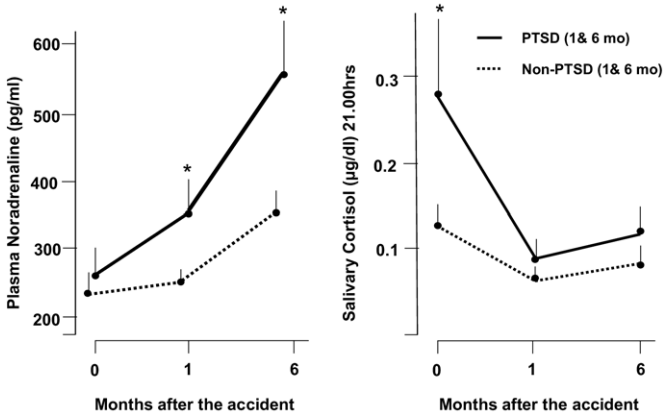

Figure 4. Plasma noradrenaline and evening salivary cortisol concentrations immediately and 1 and 6 months after motor vehicle accident in subjects who had a PTSD diagnosis at month 1 and maintained it through month 6 ($n = 8$) and those without PTSD at months 1 and 6 after the accident, respectively ($n = 23$). For noradrenaline, within-subjects effect: $p < .001$; between-subjects effect: $p = .005$. For 9 PM salivary cortisol, within-subjects effect: $p < .001$; between-subjects effect: $p < .001$.

symptoms or symptom clusters after trauma may not always fulfill all the PTSD diagnostic criteria.

Finally, salivary cortisol concentrations at noon, 6 PM, and 9 PM immediately after the accident were significantly higher in the PTSD group than in the other two groups. Cortisol levels and circadian cortisol rhythm became gradually normal in this study group (Figure 3A–C).

Longitudinally, plasma noradrenaline and evening salivary cortisol concentrations followed a divergent pattern, with noradrenaline increasing and evening salivary cortisol concentrations decreasing over the 6-month follow-up of the patients (Figure 4A and 4B).

Examining multiple comparisons (see Tables 3 and 4), the 3 × 3 mixed repeated-measures (within-subjects factor = *time* and between-subjects factor = *group*) was conducted for plasma noradrenaline and evening salivary cortisol. Regarding plasma noradrenaline, ANOVA yielded a significant main effect of *time* [$F(1.934,104.419) = 19.869$, $p < .001$, partial eta squared =

Table 3. Multiple Comparison Tests for Repeated Measures in Plasma Noradrenaline Concentrations (*p* Values) in the Longitudinal PTSD and Non-PTSD Groups

	PTSD vs. non-PTSD	PTSD vs. Control	Non-PTSD vs. Control
Immediately after the accident	*ns*	*ns*	*ns*
Month 1	.02	*ns*	*ns*
Month 6	.006	< .001	*ns*

Group	Immediately vs. Month 1 After Accident	Immediately vs. Month 6 After Accident	Month 1 vs. Month 6
PTSD	*ns*	< .001	.001
Non-PTSD	*ns*	.003	.002
Control	*ns*	*ns*	*ns*

ns, not significant.

Within-subjects $p < .001$; between-subjects $p = .005$. Subject effects: mean difference significant at the 0.05 level. Adjustment: Bonferroni correction.

Table 4. Multiple Comparison Tests for Repeated Measures 9 PM Salivary Cortisol Concentrations (*p* Values) in the Longitudinal PTSD and Non-PTSD Groups

	PTSD vs. non-PTSD	PTSD vs. Control	Non-PTSD vs. Control
Immediately after accident	.003	< .001	*ns*
Month 1 after	*ns*	*ns*	*ns*
Month 6 after	*ns*	*ns*	*ns*
Group	**Immediately vs. Month 1 After Accident**	**Immediately vs. Month 6 After Accident**	**Month 1 vs. Month 6**
PTSD	.001	.003	*ns*
Non-PTSD	.029	*ns*	*ns*
Control	*ns*	*ns*	*ns*

ns, not significant.

Within-subjects $p < .001$; between-subjects $p < .001$. Subject effects: mean difference significant at the .05 level. Adjustment: Bonferroni correction.

.269, observed power = 1] and a significant interaction between *time* and *group* [$F(3.867,104.419) = 6.599$ $p < .01$, partial eta squared = .196, observed power = .988]. Univariate tests of between-subject effects showed a significant main effect of *group* [$F(2,54) = 5.885$, $p = .005$, partial eta squared = .179, observed power = .857].

Regarding evening salivary cortisol, ANOVA yielded a significant main effect of *time* [$F(1.420,88.029) = 15.307$, $p < .001$, partial eta squared = .198, observed power = .993] and a significant interaction between *time* and *group* [$F(2.840,88.02 = 5.946$, $p < .01$, partial eta squared = .161, observed power = .940]. Univariate tests of between-subject effects showed a significant main effect of *group* [$F(2,62) = 8.859$, $p<.001$, partial eta squared = .222, observed power = .965]. The pattern of interaction between *time* and *group* for both plasma noradrenaline and evening salivary cortisol is illustrated in Figure 4.

Discussion

Noradrenaline (NA) plays major physiologic roles in arousal, attention, and memory. Dysregulation of noradrenergic neurons may be particularly involved in the hyperarousal and reexperience symptoms of PTSD (34,35). Emotional and stressful stimuli may enhance memory encoding in PTSD patients, causing excessive consolidation of memory for the stressful event and leading to the development and maintenance of intrusive thoughts, images, flashbacks, and repetitive nightmares (36,37). In our study, plasma noradrenaline was significantly elevated in patients with PTSD at both months 1 and 6 after the accident compared with the non-PTSD and control groups. Plasma noradrenaline increased progressively over time in children and adolescents with PTSD.

Evidence of increased noradrenergic activity both centrally (38,39) and peripherally has been established in adults (4–9), and children with PTSD and posttraumatic symptomatology have been reported to have increased peripheral sympathetic system activity (19,21,40,41). Thus, increased blood pressure, heart rate, and skin conductance, as well as an exaggerated startle response, have been reported in patients suffering from PTSD, reflecting increased noradrenergic activity (34). In the majority of clinical studies on PTSD, elevated urine catecholamine measurements (4–6,8,42) were indicative of increased sympathetic nervous system activity. In an early study, combat veterans with PTSD had similar plasma noradrenaline levels with healthy control subjects. However, mean plasma noradrenaline and 3-methoxy-4-hydroxyphenylglycol, a metabolite of noradrenaline, concentrations were higher in veterans with PTSD than in veterans with PTSD and comorbid depression, patients with major depressive disorder (MDD), and healthy volunteers (9). It was also reported that PTSD patients had significantly elevated noradrenaline concentrations in cerebrospinal fluid compared with normal individuals (38,39).

There is evidence for increased responsivity of noradrenergic neurons under conditions of stress in children and adults with PTSD. Hypersensitization of the noradrenergic systems may contribute to arousal symptoms in PTSD, including hypervigilance, exaggerated startle response, anger, and insomnia. Hyperarousal symptoms (criterion D) of PTSD are common in trauma survivors. It should be noted that arousal is also influenced by other neurotransmitters such as adrenaline, dopamine, serotonin, and γ-aminobutyric acid (GABA), as well as by the hypothalamic–pituitary–adrenal (HPA) and thyroid axes (34,35,37).

Because PTSD constitutes a multidimensional syndrome, specific clusters of symptoms were separately analyzed and correlated to hormone concentrations. Elevated noradrenaline was the most prominent finding examining partial PTSD diagnosis at month 6. Elevated serum or salivary cortisol was found in the majority of the clustering groups at both months 1 and 6.

Furthermore, examining the PTSD group serially, by repeated measures, we found that plasma noradrenaline was higher at the 6-month than the 1-month assessment in both the PTSD and non-PTSD groups; this elevation was independent of PTSD severity as measured by the CPTS-RI Questionnaire. According to this diagnostic instrument, PTSD severity was calculated as mean = 35.8 units at month 1 and mean = 30.3 units at month 6. These findings suggest that elevated plasma noradrenaline at month 6 reflects PTSD persistence and maintenance over time, rather than PTSD intensity.

Abnormalities in the HPA axis have been reported in PTSD adults and children; however, the interaction between this axis and the arousal–sympathetic system are often described separately rather than examined and understood in toto (43). Noradrenergic responses to stressors are associated with immediate (within minutes) responses, whereas those of the HPA axis start later and are more prolonged. In our study, as cortisol concentrations and circadian rhythm normalized in PTSD subjects at month 6, noradrenaline elevations became greater (Figure 4). This could be the result of lifting of a cortisol-mediated noradrenergic system restraint (44). Behavioral symptoms observed in PTSD patients may thus also be attributed to cortisol dysregulation that fails to shut down the catecholaminergic response in limbic structures, such as *locus caeruleus* and the amygdala, leading to PTSD development and maintenance through time.

Although the stress hormones measured here did not show clear sexual dimorphism, male and female patients, as well as prepubertal and pubertal children, were examined together, and this might have influenced our results. This was further compounded by the small number of patients suffering from PTSD. Another limitation of the present study, which was also revealed at the multiple comparison tests, was that the non-PTSD group exhibited subsyndromal PTSD, which could have partially influenced the hormonal status of this group. Another limitation of the study is that only peripheral and not the central nervous system

concentrations of hormones were measured. Nevertheless, the blood and central nervous system have some correlation for noradrenaline (45). Finally, because we did not want to burden our patients with further extensive testing, we did not use specific diagnostic instruments to screen our experimental population for comorbid anxiety disorders or depression after the accident. However, all patients were interviewed by an experienced child psychiatrist, and the process did not reveal such comorbidity.

We conclude that in children and adolescents, PTSD is associated with elevated noradrenaline and cortisol levels 1 month after a motor vehicle accident, which, diverge by 6 months after the accident, with noradrenaline increasing further and cortisol normalizing. These findings uncover a natural history of child and adolescent PTSD suggesting that in this syndrome, there is an interplay of longitudinal changes of the arousal and sympathetic systems and the HPA axis with a particular pattern characterizing those individuals who develop the syndrome. These data may explain some of the apparent discrepancies between stress hormone findings in adults with chronic PTSD in whom normal or low cortisol levels have been observed and reported.

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