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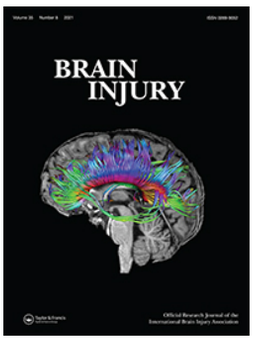

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Coping with stress before and after mild traumatic brain injury: a pilot hair cortisol study

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ABSTRACT

Background: Cortisol is a crucial hormone for adaptation to challenging and stressful situations. Hair cortisol measurement is used to determine chronic stress; the growth rate of hair allows to determine averaged cortisol levels for a longer period. *Objective*: Pre- and post-injury measures of hair cortisol were compared in patients with mild traumatic brain injury (mTBI), and related to their coping styles.

Methods: For 46 patients with mTBI, 3 cm scalp hair samples were collected 4–6 weeks post-injury, resulting in two 1 cm segments, pre- and post-injury. Hair samples were also collected for 11 healthy controls. Hair cortisol was quantified using liquid chromatography-tandem mass spectrometry (LC-MS /MS). Complaints, anxiety, depression and coping style were measured two weeks post-injury and long term (six-twelve months), added with measures for post-traumatic stress and functional outcome.

Results: There were no differences between patients' pre- and post-injury cortisol levels, nor between cortisol levels of patients and controls. However, pre- and post-injury cortisol levels of patients were negatively correlated with both passive and an avoidant coping style.

Conclusions: Our findings suggest that mTBI has no separate impact on chronic long-term cortisol levels, possibility indicating that variability in cortisol levels reflects individuals' premorbid characteristics determining coping with stress in general.

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Mild traumatic brain injury (mTBI); pre-morbid characteristics; hair cortisol; coping style; post-traumatic stress

Introduction

Traumatic Brain injury (TBI) is one of the most common neurological conditions. Patients with mild TBI (mTBI) comprise more than 80% of the total TBI population with estimated yearly incidences of 42 million people worldwide. Mild TBI implies that patients had high to maximum scores (13–15) on the Glasgow Coma Scale (GCS) which is a measure of coma and impaired consciousness, and that neuroimaging shows no or minor abnormalities. Hence, prospects for these patients are good and the majority recovers without residual sequelae. However, a small subgroup of patients (15–20%) continues to have persisting complaints that interfere with resumption of everyday life activities and work (1,2). Given the large overall incidence rates, this still concerns a substantial number of patients for whom decreased participation and loss of work productivity involve enormous societal costs.

Factors related to unfavorable outcome after mTBI

Several studies have been aimed at finding explanations for an unfavourable outcome after mTBI. A frequent finding is that acute injury-related factors, for instance GCS scores or Computed Tomography (CT) abnormalities, do not appear to be strong predictors as they explain only a small part of the variance of long-term outcomes (3,4). In contrast, indications of psychological distress, such as feelings of depression, anxiety, or post-traumatic stress, are found to be strongly associated with a negative outcome in this group (5,6). Hence, a major question is whether the ability to adequately cope with stress, such as serious life events and emotional situations, is key for successful adaptation to having sustained a mild brain injury, and decisive for being able to resume previous activities for these patients. Different styles of coping with stress can be distinguished, such as actively trying to solve problems, focusing on negative emotions, avoiding problems, or seeking social support. Depending on the situation, coping styles may be more or less adaptive, but a passive, emotion-focused style is generally considered to be maladaptive (7). Although individual preferences for specific coping styles are thought to be relatively stable, suggesting that this concerns trait-like psychological constructs, several studies suggest that coping styles might be malleable depending on the situation (8,9).

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Supplemental data for this article can be accessed on the publisher's website.

Coping with (chronic) stress in relation to mTBI

It is not clear whether employed styles of coping with stress after TBI relate to premorbid qualities of patients, or whether coping is affected by the TBI itself. Knowing whether inadequate adaptation to stress is either a premorbid personality factor or an injury-related factor determining outcome, has important implications for treatment. Usually, measurement of coping style shortly after TBI is used as an indication of the pre-injury capacity to cope with stress, but this still concerns a retrospective estimation. Consequently, there is risk of subjective bias influenced by the traumatic event. Until now, it has not been possible to obtain actual pre-injury measurements of coping style or stress regulation in this patient group. However, in the past years, the technique to measure chronic cortisol levels from hair samples has become available for clinical and research purposes, which offers an interesting opportunity to get an impression of pre-injury functioning and the interaction between stress and coping style (10). Cortisol is a hormone that is released through activation of the hypothalamic-pituitary-adrenal (HPA) axis, involved in, among others, metabolism and immune response, allowing the human body to adapt to challenging and stressful situations. Systemic cortisol levels are highly variable, and measurement of cortisol in serum, saliva or urine gives an impression only of acute or short-term stress reactions. However, hormones are also retained in hair which allows measurement of long-term exposure to cortisol. It has been found that somatic conditions, such as diabetes mellitus, obesity, heart failure but also Cushing's syndrome or use of exogenous glucocorticoids influence hair cortisol levels (10). However, a large range of studies provided evidence that also mental stressors and psychopathology affect hair cortisol levels, strongly suggesting that this may be a valid indication of chronic psychological stress. A systematic review of Staufenbiehl and colleagues, comprising 19 studies, showed that long-term alterations of the HPA axis result into changed levels of cortisol and are associated with chronic stressful situations, and psychopathology, and consequently, with adverse long-term health outcomes (11). Since chronic stress can be defined as a state in which stressors continuously exceed a subject's ability to cope, chronically altered cortisol levels are likely to reflect personal factors as well, that is, the extent to which subjects are able to adequately cope with and adapt to stress. Altered hair cortisol levels have been found in people who sustained serious life events, had severe chronic pain, were unemployed, or had serious mental disorders such as major depressive disorder or Post-traumatic Stress Disorder (11). Hair cortisol analysis not only offers the possibility to measure long-term cortisol as indicator of chronic stress, but also to compare several hair segments to each other allowing the establishment of a timeline. Since hair grows at a relatively constant rate of 1 cm a month, hair samples that are collected timely and are of sufficient length will include segments before the presence of a stressful event (10). This offers the unique possibility to measure stress levels from before having sustained a mTBI and to determine whether this event had effect on coping with stress. Hence, the cortisol level in a pre-injury hair sample is considered a promising biomarker for a patient's stress burden in a past period. Sörbo and colleagues were the first to study pre- and post-injury hair cortisol levels in a group of patients who sustained either a severe TBI or an aneurysmal subarachnoid haemorrhage (SAH), finding a possible relation with reported stress before the brain injury (12). The question remains whether this relation can also be found in patients who sustained a mild TBI.

Study aims

The present study concerns a sub-study of the UPFRONT study (13). To date, this is the first study in which both pre- and post-injury chronic cortisol levels were determined in patients with a mild TBI and were related to coping style and long-term functioning. Previous studies found conflicting results with respect to altered salivary cortisol levels to be related to long-term depression, fatigue and chronic stress in patients with mTBI (14,15). However, chronic hair cortisol has not been investigated, nor the possible moderating role of coping style. Since our assumption was that chronic cortisol reflects a relatively stable individual way of coping with stress over time, we hypothesised that chronic cortisol would be related to measures of coping style. Furthermore, we examined whether these cortisol levels would also be related to indications of long-term functioning after TBI, such as psychological distress and well-being, when the acute stress related to sustaining the injury would have disappeared. We were particularly interested in a possible relationship of cortisol levels and coping style with post-traumatic stress and long-term outcome, that is six or more months post-trauma.

Material and methods

Design and setting

This study was part of a larger prospective cohort multicentre study investigating predictors for long-term outcome in adult mTBI (the UPFRONT-study) that ran from January 2013 to January 2017 (13). Data collection for the current study was done in only one of the participating centres, the University Medical Center Groningen (UMCG), a level-I trauma centre. Patients included in the overall UPFRONT-study had to be at least 16 years of age and had to have a confirmed diagnosed mTBI, with loss of consciousness (LOC) of max 30 minutes and Glasgow Coma Scale (GCS) scores ranging from 13 to 15. On admission, GCS scores were determined and a CT scan of the brain was performed. CT abnormalities were assessed by a radiologist at the Emergency Department and rated as 1. no traumatic abnormalities or 2. traumatic abnormalities. Patients who agreed to take part in the study filled out questionnaires at 2 weeks and at 3, 6 and 12 months post injury; at 6 and 12 months outcome was determined. For the present study, only measurements at 2 weeks, 6 months and, if data from the 6-month time point were missing, 12 months were used.

Participants

Within the UPFRONT-study, a part of the patients in the age range 18–65 years were asked to take part in a sub-study involving advanced structural and functional neuroimaging

(16); these same patients were asked to participate in the current study and were included if they consented to donate a hair sample. Excluded were patients who had insufficiently long scalp hair, patients who had medical conditions that might affect cortisol production (such as Cushing's syndrome) or patients who used medication or beauty products containing hydrocortisone. In addition, a group of 11 healthy controls, who had agreed to participate in the neuroimaging sub-study and complied to the same exclusion criteria (with as addition: not having sustained any brain injury) were asked to donate a hair sample.

The Medical Ethical Committee of the University Medical Center Groningen approved of the UPFRONT-study and its sub-studies. All patients gave informed written consent to participate in compliance with the Helsinki Declaration.

Hair cortisol: collection and processing

At 4–6 weeks post injury, samples of scalp hair of 3 cm or longer were collected from the posterior vertex as close as possible to the scalp, following the procedure as described in (10). Scissors were used to cut a lock of hair as thick as a pencil. Post-processing of these samples took place in the Erasmus MC, Rotterdam, the Netherlands. For each patient, the hair sample was divided into a distal 1.0 cm pre-injury segment starting 1.5 cm before the estimated hair position corresponding the moment of injury and a proximal 1.0 cm post-injury segment. For the healthy controls, two similar segments (i.e. 0 to 1.0 cm and 1.5 to 2.5 cm from the scalp) were analysed. Further processing of the hair segments was performed as described in (17). In short, after weighing and washing the hair samples were incubated for 18 hours with 1.4 mL LCMS-grade methanol and 100 µL internal standard of deuterated cortisol for steroid extraction. Extracted samples were eventually purified with solid-phase extraction and subsequently quantified for cortisol levels with liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Xevo TQ-S system (Wates, Milford, MA, USA). This resulted in two variables: Pre-trauma cortisol and Post-trauma cortisol.

Questionnaires

Questionnaires were administered in the patient group only. At 2 weeks post-injury, questionnaires for Coping style, Post-traumatic complaints, Depression and Anxiety were administered. At 6 and 12 months these same questionnaires were administered, together with measures for Post-traumatic Stress and Outcome.

Coping style

The Dutch Utrechtse Coping Lijst (UCL) was used to measure coping style at 2 weeks and 6 months post-injury (18). The UCL is a 47-item questionnaire with seven subscales that represent different coping styles. Items are rated on a scale from 1 (seldom or never) to 4 (very often). The scales represent the following coping styles: (UCL-Act: active problem-focused coping style (seven items; max score 28), UCL-Pas: passive emotion-focused coping style (7 items, max score 28); UCL-Pall: palliative distraction-seeking coping style (8 items: max score 32); UCL-Avoi: problem avoidant coping style (8 items: max score 32); UCL-Sup: social support seeking coping style (6 items: max score 24); UCL-Exem: expression of emotions involving coping style (3 items: max score 12) and UCL-Pos: positive reframing coping style (5 items: max score 20). Higher scores indicate higher use of that specific coping style. The UCL had good psychometric properties. Internal consistency of the scales was found to be moderate to high (Cronbach's Alpha 0.67 to 0.84 (19)).

Post-traumatic complaints

The Head Injury Symptom Checklist (HISC) (20,21) was used to measure post-traumatic complaints at 2 weeks and 6 months post-injury. The HISC contains 21 frequently reported complaints after TBI each rated on a scale from 0 to 2 resulting in a sum score of current complaints corrected for pre-injury levels with a maximum of 42 (20).

Depression and anxiety

The presence or absence of depression and/or anxiety at 2 weeks and 6 months post injury was assessed with the Hospital Anxiety and Depression Scale (HADS) (22). The HADS is a 14-item questionnaire, containing two 7-item subscales, the depression scale (HADS-D) and the anxiety scale (HADS-A). Items are rated on a scale from 0 to 3, with maximum scores for each subscale of 21. Higher scores indicate higher levels of anxiety or depression, respectively. The HADS has good psychometric properties. Internal consistency of both scales was found to be moderate to high (Cronbach's Alpha HADS-D 0.67 to 0.90; HADS-A 0.68 to 0.93) (23).

Post-traumatic stress

The Impact of Event scale (IES) is a self-reported measure of post-traumatic stress, administered at 6 months post injury (24). The IES consists of 15 statements, with scores ranging from 1 to 5 and a maximum score of 75. A higher score indicates a higher level of post-traumatic stress. The IES has good psychometric properties. Internal consistencies were found to be moderate to high (Cronbach's Alpha 0.65 to 0.92) (25).

Functional outcome

The extended version of the Glasgow Outcome Scale (GOSE) (26,27) can be used as a structured interview that patients can fill out at home. It provides eight categories of outcome, ranging from 1 indicating "death", to 8 indicating "upper good recovery". A GOSE score of 8 indicates a return to fully functioning status at daily activities and work without any disabilities. GOSE scores determined at 6 months post injury were used, but in case these data were missing, GOSE scores determined at 12 months were used.

Statistical analysis

Data were analysed using SPSS (version 24.0). Demographic variables of the mTBI and healthy control groups were compared using parametric (Student's *t*-test) and non-parametric (Mann–Whitney *U*-test, χ^2-test) testing when appropriate. Because the distribution of cortisol values, expressed as

picogram (pg) per milligram (mg) hair, was highly skewed, a log10 transformation was applied and these transformed variables were used for the analyses. Non-transformed values exceeding 100 pg/mg were considered as outliers and removed. Paired sample t-tests were used to compare pre-injury and post-injury cortisol levels of the patients with mTBI, and two sample t-tests to compare the mTBI and healthy control groups at each time point. Cohen's *d* effect sizes were computed (for two sample t-tests: $d = \frac{\overline{X}1-\overline{X}2}{\sqrt{(SD1^2+SD2^2/2)}}$; for paired sample t-tests: $d = \frac{t}{\sqrt{N}}$) (28). Repeated measures analysis (GLM) was applied to compare pre-injury and post-injury cortisol levels of the mTBI group to cortisol levels of healthy controls, in particular, for interaction effects indicating possible differences between the groups regarding the pattern of change over time. Spearman correlations were calculated to determine the associations of pre- and post-injury cortisol levels (i.e., dependent variables) of patients with 2 weeks and 6 month indications of post-traumatic complaints, coping styles and emotional distress, and with 6 month indications of post-traumatic stress and outcome (i.e., independent variables). Regarding coping style, our main focus was on active, passive and avoidant coping styles, which is in line with other research (13). Analyses for the other four coping styles were added as a data supplement. For the measures for emotional distress (HADS), complaints (HISC), coping style (UCL), post-traumatic stress (IES) and outcome (GOSE), missing values for four patients at 6 months were replaced by values obtained at the 12 month measurement. Overall alpha was set at 0.05. Since this was a pilot study, and the variables of interest were not fully independent, we did not correct correlation *p*-values for multiple comparisons.

Results

Group characteristics

Table 1 shows demographic characteristics and Log 10 transformed pre- and post-injury cortisol levels of both patients and controls, as well as injury-related data of the patients with mTBI.

Of the 68 patients with mTBI who took part in the neuroimaging sub study, 46 patients fulfiled the inclusion criteria and were willing to take part in the hair cortisol study. After weighing and processing the pre- and post-injury hair samples, three patients were excluded for: both samples did not yield sufficient cortisol (n = 1) or because cortisol levels exceeded the level of 100 pg/mg (n = 2). Hence, data of 43 patients could be analysed; for 42 patients pre- and post-injury cortisol measures were collected, and for 1 patient only post-injury cortisol measures were available. For all of the healthy controls, cortisol measurements for two time points were available.

Table 1. Demographical characteristics and mean cortisol values of mTBI patients and controls, injury-related characteristics of mTBI patients.

Variable	mTBI	Healthy controls
	n = 43	n = 11
Male, n (%)	26 (60.5%)	7 (63.6%)
Age, M (sd, range)	38.8 (16.5, 19–64)	36.7 (14.2, 18–61)
Education, median (range)	6 (3–7)	6 (5–7)
GCS score, median (range)	15 (13–15)	
CT abnormalities, n (%)	5 (11.6%)	
Injury mechanism, %:		
Traffic/Falls/Violence/Other	42/44/5/9	
Loss of consciousness, yes, n (%)	35 (81.4%)	
Post-traumatic amnesia, yes, n (%)	36 (83.7%)	
Pre- cortisol Log10, M (sd, range))	0.66 (0.43, -0.19–1.60)	0.63 (0.42, 0.08–1.48)
Post-cortisol Log10, M (sd, range)	0.70 (0.34, 0.06–1.64)	0.68 (0.35, 0.18–1.47)

Abbreviations: GCS, Glasgow Coma Scale.

Statistical testing showed that the patients with mTBI were well-matched to the healthy controls regarding sex distribution ($\chi 2 = 0.037$, $p = .847$), age ($t = -0.38$, $p = .709$) and educational level ($t = 0.20$, $p = .839$).

Two patients (4.7% of the group) reported pre-existing psychiatric conditions; one suffered from anxiety disorder, the other from depression. Pre- and post-injury cortisol levels of the patient with anxiety were within the range of the mTBI group; those of the patient with depression were the highest of the mTBI group. Therefore, the cortisol analyses were also conducted after exclusion of patients with pre-injury depression. Given that brain injury as result of traffic incidents or violence may have a different psychological impact than falls, we tested whether these groups differed with regard to cortisol levels: this was not the case (Pre-trauma cortisol ($t = 1.3$, $p = .143$); Post-trauma cortisol ($t = 1.9$, $p = .062$)). Also, we tested whether there was a difference between men and women, which was not the case either (Pre-trauma cortisol ($t = 1.7$, $p = .106$); Post-trauma cortisol ($t = 0.7$, $p = .516$)).

Cortisol measures: differences between patients with mTBI and healthy controls

Paired samples t-tests were performed for the two cortisol measures of the mTBI and control group separately. For the patients with mTBI, the difference between the pre-injury and post-injury cortisol measurement was not significant ($t = -1.89$, $p = .066$, $d = -0.29$); pre- and post-injury measures correlated highly (Spearman rho = 0.96, $p < .001$). For healthy controls, the difference between both cortisol measures was also not significant ($t = -1.56$, $p = .151$, $d = -0.47$), with also a very high correlation between measures (Spearman rho = 0.98, $p < .001$). T-tests showed that there were no significant differences between the patients with mTBI and the healthy controls for the pre-injury/first cortisol measurement ($t = -0.196$, $p = .846$, $d = -0.067$) nor for the post-injury /second cortisol measurement ($t = -0.158$, $p = .875$, $d = -0.053$). Repeated measures analysis showed no overall effect of time ($F(1,51) = 3.4$, $p = .070$) or group ($F(1,51) = 0.047$, $p = .829$), nor an interaction effect over time ($F(1, 51) = 0.00$, $p = .989$).

Results remained consistent after exclusion of the patient with pre-injury depression.

Results of questionnaires for patients with mTBI at 2 weeks and 6/12 months

Table 2 shows the mean scores, standard deviations and ranges of the variables that were measured at 2 weeks and 6 or 12 months. For the 6 or 12 month measures, all questionnaire

Table 2. Questionnaires at two weeks and 6/12 months for the mTBI patients.

mTBI	Two weeks	6/12 months
	n = 43	n = 40
HISC	6.8 (*5.5*, 0–17)	5.8 (*5.4*, 0–18)
HADS-A	4.5 (*3.8*, 0–16)	4.4 (*3.8*, 0–15)
HADS-D	4.0 (*4.2*, 0–17)	3.2 (*3.8*, 0–15)
UCL-Active	19.0 (*2.7*, 13–24)	18.5 (*3.3*, 10–24)
UCL-Passive	10.5 (*2.3*, 7–16)	10.9 (*3.0*, 7–18)
UCL-Avoidant	16.3 (3.1, 10-21)	13.8 (3.3, 7-22)
IES		10.0 (*13.9*, 0–56)
GOSE, median (range)		8 (4–8)

Abbreviations: HISC, Head Injury Symptom Checklist; HADS-A, Hospital Anxiety and Depression Scale- Anxiety Scale; HADS-D, Hospital Anxiety and Depression Scale-Depression Scale; UCL-Active, Utrechtse Coping Lijst- Active scale; UCL-Passive, Utrechtse Coping Lijst- Passive scale; IES, Impact of Event Scale; GOSE, Glasgow Outcome Scale Extended.
Values represent means (SD, range), unless stated otherwise.

data were missing from three patients, and for additional two patients for the HADS, IES, UCL and HISC questionnaires only.

Associations between cortisol measures and questionnaires for patients with mTBI

Table 3 shows the Spearman correlations for the patients with mTBI between both cortisol measures and the 2 week measures for post-traumatic complaints (HISC), post-traumatic distress (depression HADS-D, anxiety HADS-A) with the coping styles of interest. Analyses for the other coping styles can be found in the data supplement. The correlations of both cortisol measures with all other measures are low and non-significant, except for UCL-Pas at 2 weeks, which was moderate and significant. In addition, use of an avoidant coping style (UCL-Avoi) showed a significant correlation with chronic cortisol level pre-injury. These correlations were negative, indicating that a higher use of a passive (UCL-Pas) or an avoidant (UCL-Avoi) coping style at 2 weeks was related to lower chronic cortisol levels. These results remained consistent after exclusion of patients with pre-injury depression. In addition, use of a passive coping style was significantly positively related to level of anxiety. Furthermore, there were mutually significant and high positive correlations between anxiety, depression and post-traumatic complaints 2 weeks post injury.

Table 3. Spearman correlations between cortisol values and scores on questionnaires at two weeks for the mTBI patients.

Variables	1 PreTrCort	2 PostTrCort	3 HISC-2 w	4 HADS-A2w	5 HADS-D2w
2. PostTrCort	.96*				
3. HISC-2 w	-.05	-.01			
4. HADS-A2w	-.09	-.11	.75*		
5. HADS-D2w	-.06	.04	.85*	.67*	
6. UCL-Act2w	.09	.02	.04	.01	-.09
7. UCL-Pas2w	-.34*	-.37*	-.24	.46*	.31
9. UCL-Avoi2w	-.37*	-.30	-.11	-.05	-.04

Abbreviations: PreTrCort, pre-trauma hair cortisol level Log10 transformed; PostTrCort, post-trauma hair cortisol level Log10transformed; HISC-2 w, Head Injury Symptom Checklist at 2 weeks; HADS-A2w, Hospital Anxiety and Depression Scale- Anxiety scale at 2 weeks; HADS-D2w, Hospital Anxiety and Depression Scale- Depression scale at 2 weeks; UCL-Act2w, Utrechtse Coping Lijst- Active at 2 weeks; UCL-Pas2w, Utrechtse Coping Lijst- Passive at 2 weeks; UCL-Avoi2w, Utrechtse Coping Lijst-Avoidant at 2 weeks.
* = $p < 0.05$.

Table 4 shows the Spearman correlations for the mTBI group between both cortisol levels and the 6 month measures for depression and anxiety (HADS-D, HADS-A), post-traumatic complaints (HISC), coping styles as well as post-traumatic stress (IES) and outcome (GOSE). Both a passive (UCL-Pas) and an avoidant (UCL-Avoi) coping style at 6 months were significantly, though negatively, correlated to both pre- and post-trauma cortisol levels. A higher use of a passive coping style, but not of an avoidant coping style, was significantly related to more post-traumatic complaints and higher levels of anxiety and depression at 6 months post injury (which also showed mutually significant correlations), but there were no significant direct correlations of both cortisol measures with these latter variables. In addition, post-traumatic stress (IES6m) and outcome showed (GOSE) significant correlations with the measures for post-traumatic complaints, anxiety and depression, as well as with each other. The latter finding indicated that lower levels of post-traumatic stress were related to a better outcome.

Discussion

This is the first study on mild TBI that determined chronic hair cortisol from a one-month period just before sustaining the brain injury, as a pre-injury measure of chronic stress, and compared this to both post-injury hair cortisol as well as to patients' employed coping styles. This is very relevant since there is a long-standing debate regarding the extent to which pre-injury characteristics are decisive for long-term outcome and recovery from this type of brain injury. Until now, measures of stress and coping could only be determined post-injury, for instance by means of questionnaires asking for retrospective evaluations to estimate pre-injury functioning. However, such estimates may be incorrect as it is not clear to which extent such post-injury rated measures are biased by the brain injury itself or its psychological impact. Chronic hair cortisol has been found to be a reliable measure for chronic stress, given that other factors such as physical conditions or use of medication that may affect cortisol levels are controlled for, which we did in our study. To date, there is only one recent study that investigated pre-injury cortisol levels after brain injury, albeit in different groups, namely patients with severe TBI and aneurysmal subarachnoid haemorrhage (SAH) (12). This study found a relation of pre-injury cortisol levels with having sustained stressful events pre-injury. Another study in patients with aneurysmal SAH found significant correlations between post-injury hair cortisol and psychological problems and poor sleep, corroborating the merit of hair cortisol as a measure of chronic stress in populations with brain injury (29).

Our study showed for healthy control participants that measurements of chronic cortisol at two different time points were highly correlated, indicating that measurement of hair cortisol is very stable over time in normal circumstances. In our patient group with mild TBI we found also no significant difference between the pre-injury and post-injury chronic hair cortisol levels, with a high correlation between both measures as well. Sustaining a brain injury is a stressful event and it seems likely that this would be reflected in an altered cortisol

Table 4. Spearman correlations between cortisol values and scores on questionnaires at 6/12 months for the mTBI patients.

Variables	1 PreTrCort	2 PostTrCort	3 HISC6m	4 HADS-A6m	5 HADS-D6m	6 IES6m	7 GOSE6m
2. PostTrCort	.96*						
3. HISC6m	.04	.04					
4. HADS-A6m	.10	.05	.67*				
5. HADS-D6m	.01	-.03	.89*	.73*			
6. IES6m	.31	.32	0.54*	.59*	.56*		
7. GOSE6m	-.01	-.03	-.67*	-.53*	-.69*	-.40*	
8. UCL-Act6m	.13	.17	-.16	-.13	-.29	-.20	.16
9. UCL-Pas6m	-.36*	-.39*	.36*	.38*	.41*	.29	-.30
11. UCL-Avoi6m	-.45*	-.39*	.24	.05	.28	-.10	-.14

Abbreviations: PreTrCort, pre-trauma hair cortisol level Log10 transformed; PostTrCort, post-trauma hair cortisol level Log10transformed; HISC6m, Head Injury Symptom Checklist at 6 months; HADS-A6m, Hospital Anxiety and Depression Scale- Anxiety scale at 6 months; HADS-D6m, Hospital Anxiety and Depression Scale- Depression scale at 6 months; IES6m, Impact of Event Scale at 6 months; GOSE6m, Glasgow Outcome Scale Extended at 6 months. UCL-Act6m, Utrechtse Coping Lijst-Active at 6 months; UCL-Pas6m, Utrechtse Coping Lijst- Passive at 6 months; UCL-Avoi6m, Utrechtse Coping Lijst-Avoidant at 6 months.
* = $p < 0.05$.

level in the acute post-injury period. Various studies, including our own, have demonstrated that after mTBI many patients continue to experience high levels of (post-traumatic) stress; however, none of these studies was able to compare with pre-injury stress (5,6,11). The chronic cortisol level determined in a 1 cm hair sample is an average covering a period of four weeks post-injury. Based on our findings that the post-injury level did not differ in the patient group from the pre-injury hair cortisol level (and was even very highly correlated), we conclude that post-injury cortisol covering a one-month period seems not to be altered by the traumatic event or the brain injury itself. Possibly, the period of acute stress (both the mental stress of the traumatic event as well as pain as physical stressor) is relatively short, and when adequate pain control is established, and the acute mental stressors subside, the amount of total stress is not chronic enough to be reflected in a long-term measurement of cortisol level. Moreover, comparison of both measurements of the patients with mTBI with those of the healthy controls revealed that at both time points there were no significant differences between the groups, nor was there any indication that there was a different trajectory over time for the patients with mTBI. Hence, measurement of chronic cortisol at different time points in patients and healthy controls shows that levels are very stable, even when a traumatic event has occurred in between two measurements as is the case for the patients with mTBI. Therefore, we tentatively conclude that overall, a mild TBI has no impact on chronic stress levels as reflected in chronic hair cortisol. This would imply that individual variability in these cortisol levels mainly reflects individual's premorbid characteristics, part of which pertains to their abilities to cope with stress. Consequently, pre-injury cortisol level may be considered a biomarker that reflects the extent to which individuals succeeded in coping with stress.

Within the mTBI group, we analysed whether individual variability in chronic cortisol levels was associated with different coping styles as well as with indications of both short and long-term post-TBI psychological distress (mood, anxiety, post-traumatic complaints) and long-term post-traumatic stress and outcome. We found significant, moderately high, correlations of both pre- and post-injury cortisol level with the use of a passive coping style, both at 2 weeks and at 6–12 months post injury. Use of an avoidant coping style was also at both time points significantly related to Pre-injury cortisol levels, and at 6–12 months also with Post-injury cortisol level. Both styles of coping, passive and avoidant, are generally considered to be maladaptive, although avoiding problems may be adaptive in certain situations. However, a passive coping style, involving ruminating, catastrophizing and focusing on negative emotions, is always conceived to be an inadequate way of dealing with stress. Passive coping likely results in insufficient adaptation to the changed situation and contributes to the persistence of problems. This is corroborated by the significant correlations that were found between preference of a passive coping style at both 2 weeks and 6–12 months, and high levels of complaints, anxiety and depression. The present study concerned a small subgroup of the large group of patients with mTBI that were followed in the UPFRONT-study with the aim to find predictors for long-term outcome. In a recent publication (13) we reported that high use of a passive coping style was one of the significant predictors for outcome at six months post-injury. In the study of Scheenen, we found, when comparing all coping styles over time in the total patient group of the UPFRONT-study, that use of a passive coping style was very stable over time (30). This suggests that neither the occurrence of the brain injury itself nor the subsequent recovery stage have influence on patients' preference for this type of coping with stress. In our present study, we found that preference for a passive coping style is related to chronic cortisol level as substantiated by the significant correlation with post-injury cortisol. Moreover, given the almost similar significant correlation with pre-injury cortisol level, we believe strongly that this can be interpreted as an indication that preference for a passive coping style was already present before the TBI was sustained. Hence, this suggests that use of a passive coping style is a stable personality characteristic which is reflected in HPA axis dysregulation expressed as stable, lower chronic cortisol levels. In addition, in the study of Scheenen (30), it was found that use of an avoidant coping style was not stable over time, showing a decrease over six months. Correlations of avoidant coping with both pre- and post-injury cortisol levels were higher for the six-month measurement than for the two weeks measurement, suggesting that this relation was stronger for the group that continued to apply this coping style over time.

Measures of complaints, anxiety and depression were highly related to each other at both time points. At two weeks,

a passive coping style was related to anxiety only, but at six months passive coping was related to all three measures. Although correlation analysis allows no conclusions regarding causal relations, it seems plausible that a pre-existing maladaptive style of coping with stress and emotional problems will result in higher levels of complaints and psychological distress after a traumatic event such as a mild TBI. This seems even more likely because use of an avoidant coping style, which is not necessarily maladaptive, was not significantly related to complaints, anxiety and depression at both time points. Since we found no direct relationship between cortisol levels and psychological distress (anxiety and depression) but did find a passive coping style to be significantly correlated to both types of measures, this may indicate an indirect relation between psychological distress and altered cortisol levels, possibly mediated by a passive coping style. Indications of long-term complaints, anxiety and depression were significantly correlated to the presence of long-term post-traumatic stress as well as to an unfavourable outcome. Also, long-term post-traumatic stress levels were significantly related to an unfavourable outcome. However, correlations of long-term post-traumatic stress with indications of inadequate coping such as a passive coping style and pre-injury cortisol levels were non-significant though moderate.

An unexpected finding concerned the direction of the relationship between both a passive as well as an avoidant coping style with chronic hair cortisol, since we found that higher use of both coping styles was related to lower levels of chronic hair cortisol. In the systematic review of Staufenbiehl (11) the majority of studies that described levels of hair cortisol in relation to chronic stressors found that stressful conditions went together with higher levels of hair cortisol, such as regarding shift-workers (31), unemployment (32), chronic pain (33) and presence of major life stressors (34). Similarly, in several studies investigating the relationship between hair cortisol and mental illness, higher hair cortisol levels were found, such as with depression (35,36) and withdrawal of alcoholism (37). Nevertheless, also lower hair cortisol levels were found in some studies, mainly in anxiety-related disorders. For instance, Steudte and colleagues found significantly lower levels of hair cortisol in patients with generalised anxiety disorder (GAD), suggesting hypocortisolism (38). Subsequently, circumstantial evidence for hypocortisolism in patients with PTSD and traumatised controls, with lower long-term cortisol levels in comparison to non-traumatised controls was found (39). A meta-analysis of Stalder and colleagues concluded that stress-exposure in general was likely to result in higher hair cortisol, in particular when the stress was still ongoing, but that anxiety induced disorders such as PTSD were more likely to result in lower levels of hair cortisol (40). However, in a recent study of Steudte and colleagues, it was found that only patients who had both generalized anxiety disorder and concomitant major depression showed endocrine dysregulation resulting in lower chronic cortisol levels (41). These findings contrast reports regarding short-term cortisol concentrations, such as measured in salivary secretion or serum, that showed an increase in patients with depression and with anxiety (42). A possible explanation might be that short-term cortisol levels change in reaction to momentary stressors, to which these patients are apparently still sensitive, indicating an ongoing physiological response. However, a decreased chronic level of cortisol might be an indication of more general behavioural tendency to encounter as little as possible situations that might elicit stress, which one would expect as a consequence of employing either a passive or an avoidant coping style. An alternative explanation is that chronic cortisol dysregulation reflects a relative hypersensitivity of the glucocorticoid receptor, as has been shown to be partially genetically determined, and may lead to increased cortisol action at the tissue level, but relatively low circulating cortisol concentrations due to increased negative feedback of the HPA-axis (43).

There are some limitations that have to be mentioned. First, the patient sample was relatively small and heterogeneous, affecting the power of this study. Second, although patients with cortisol levels exceeding 100 pg/mg were considered to be outliers, of whom hair cortisol levels probably have (unknowingly) been influenced by use of products containing glucocorticoids, we can still not be sure that in the remaining patient group with lower hair cortisol levels these factors might have been of influence, although to a lesser extent, confounding our results. Another point that should be considered is that our results are based on the assumption that hair grows with a relatively stable rate of 1 cm a month. However, a study of LeBeau and colleagues (2011) emphasises that growth variability, despite small, may still influence results. Also, Wester and van Rossum stress that this should be considered when creating retrospective timelines (10). Therefore, we used a sufficiently long hair sample and did not use the middle section of 0.5 cm, to be sure that the distal 1 cm section of 1.5 to 2.5 cm surely represented the pre-injury period (44). Moreover, we analysed hair samples from the posterior vertex which is the preferred place with respect to hair analysis given among others the relatively consistent growth rate (45). In addition, we used an LC-MS/MS -based technique to quantify cortisol levels, which is the state-of-the art method in endocrine research. Finally, no data regarding complaints, mood, coping, and lifetime stressors were gathered from healthy controls, as they were primarily included for the neuroimaging part of the UPFRONT study. This limits our conclusions about stress in this group, as well as the comparison with the patient group.

In conclusion: In this pilot study, we found a significant negative correlation of both pre- and post-injury cortisol levels with long-term use of a passive coping style, and, to a lesser extent, use of an avoidant coping style, in the group of patients with mild TBI. Interestingly, cortisol levels showed no direct relations with indications of psychological distress post-injury. However, these were in turn related to a passive coping style, but not to use of an avoidant coping style. This suggests that a passive coping style reflects a longer existing tendency to engage as little as possible in situations that induce a biological stress response because of a lack of psychological and/or physiological resources to deal adequately with such situations. This tendency may be reflected in decreased chronic cortisol levels indicating either hypo activation of the HPA axis, or a hypersensitivity of the glucocorticoid receptor, which could lead to increased cortisol effects at the cellular level throughout the body and brain. At the same time, we deem it highly likely

that not engaging in challenging situations may not be helpful to recover adequately from a mTBI, in particular when patients are inclined to ruminate and focus on negative emotions, which may lead to higher levels of complaints and psychological distress. Still, just employing an avoidant coping style without worrying may also lead to lower chronic cortisol levels, but may still be an adaptive way of dealing with stress. Taking the effects of different coping styles into account may have important implications for treatment and rehabilitation.

Declaration of interest statement

JMS, JvdN, EFCR, HJH, MES, MEK, MS and TL declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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