



Review

The relationship between cortisol, stress and psychiatric illness: New insights using hair analysis



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ABSTRACT

Background: Stress is an established important contributor to the development of mental illness and stress related disorders. The biology implicated in the homeostasis of pathological stress mechanisms is not fully established. One of the difficulties with current techniques is the limitation in capturing chronic levels of cortisol as an expression of stress levels in humans. Hair samples can be used to evaluate cortisol levels averaged over relatively long periods of time, therefore providing a more valid measure of chronic levels of this hormone. A highly replicable technique to measure long-term cortisol could prove pivotal in improving our understanding of the role of stress in psychiatric disorders.

Methods: This review synthesises all the published studies relating hair cortisol concentration (HCC) to stress and to psychiatric disorders. It describes and summarises their findings with the aim of providing a summary picture of the current state of this line of research.

Results: The strongest finding to date is the replicable increases in hair cortisol associated with stressful life events. Findings in psychiatric disorders are more sparse and inconsistent. There is some support for the presence of raised HCC in major depressive disorders, and for lowered HCC in posttraumatic stress disorder, suggesting chronic hypercortisolaemia and hypocortisolaemia respectively.

Conclusions: HCC is a promising methodology to study chronic cortisol levels with the potential to help characterise psychiatric and stress related disorders. The combination of chronic and acute cortisol measurements has the potential for more accurately determining different aspects of the stress response, and ultimately for the development of a biological marker to aid diagnosis and response to treatment.

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1. Background

The reaction of the brain to stress includes activation of the hypothalamic-pituitary-adrenal (HPA) axis. This axis consists of a chain of stimulatory hormones and feedback loops under the control of higher cerebral centres determining its overall activity (Papadopoulos and Cleare, 2012). Cortisol, the steroidal hormone of this axis, is at the centre of a pathophysiological stress response. This response can be either normal (adaptive) or abnormal (maladaptive) (Selye, 1946). A maladaptive response is often associated with extreme stress conditions both in terms of duration of exposure and force of the stressor (Gozhenko et al., 2009). When the ability of the individual to sustain stress is overcome a maladaptive stress response can lead to exhaustion and this can be measured.

Historically the most commonly used methodologies to measure cortisol levels have included samples of serum, saliva, and urine. These sampling methods are particularly effective in measuring acute levels of cortisol. Chronic levels are more difficult to measure and have required collecting multiple samples at many different time points (Pruessner and Kirschbaum, 2003). This approach has resulted in significant variability in the findings mostly due inherently high variability across samples potentially obscuring longer term alterations (Kudielka, 2003; Miller et al., 2007; Warnock et al., 2010). To bypass this limitation a recent method for measuring chronic concentration has included sampling cortisol in hair. The validity of this method, which consists of collecting one sample of hair to cover a longer period of cortisol synthesis, has been investigated in animal models and healthy individuals. Research studies have investigated the correlation between hair cortisol concentration (HCC) in a single sample of hair with a variable number of specimens collected using an alternative sample type. Davenport et al. (2006) found that HCC correlated with 8 saliva samples over a 2 week period in Rhesus monkeys. In healthy humans, Xie et al. (2011) measured 3 saliva samples over a 1 week period and found them to correlate with HCC. D'Anna-Hernandez et al. (2011) found this association in pregnant women, and van Holland et al. (2012) in construction workers. Other similar research utilising other specimens have yielded similar results. Sauvé et al. (2007), for example found that 24-h urinary cortisol correlated significantly with HCC differently from single point saliva or serum assessments. Overall these and other results suggest that HCC reflects long-term cortisol secretion, with the strength of this association increasing with the number of correlating samples (Stalder and Kirschbaum, 2012).

A number of specimens including saliva, urine or blood have been utilised to measure chronic levels of cortisol in a number of psychiatric conditions to understand how HPA axis activity differs in comparison with healthy controls (Russell, 2012). Results have been predictably variable so that more recently HCC has

increasingly been used to more effectively measure chronic cortisol levels. Obtaining a long-term cortisol secretion pattern at different points of a psychiatric disorder, such as its development, maintenance and resolution, is an important step in increasing the diagnostic specificity and the categorisation of symptom cluster subtypes. Furthermore, such patterns of cortisol response may have clinical utility when trying to corroborate evidence of response to therapeutic interventions (Fisher and Stoolmiller, 2007). A recently published review (Staufenbiel et al., 2012) was instrumental in highlighting the importance of hair cortisol analysis in psychiatry. Here we present the most up to date and inclusive systematic review published since Staufenbiel et al.'s work by including twice as many reports available in the literature evaluating hair cortisol levels in stress-related conditions and psychiatric disorders. We also offer a view on the significance of this type of research in the development of biological markers in psychiatry, a currently topical research focus in the field.

2. Methods

A comprehensive systematic search was conducted from January 1978 to March 2015 to identify all relevant studies and included the following databases: Pubmed, Embase, Ovid MEDLINE(R), PsycINFO, PsycARTICLES. The first research report identified that met the inclusion criteria was published in 2007 (Yamada et al., 2007). Search terms were: "Long term cortisol" OR "hair cortisol" AND "Psychiatric OR depress*" OR "affective disorder" OR "psychosis OR bipolar*" OR "personality disorder" OR "eating disorder" OR "mental health". Inclusion criteria were: a) research was conducted in humans; b) the study used scalp hair from the posterior vertex (this measure has in fact been associated with the least variance between different strands, e.g. Sauvé et al., 2007); c) provided sufficient information regarding sampling and cortisol extraction methods; d) stress and/or diagnosis were systematically evaluated; and e) studies were case-control comparisons. Data repetition was avoided by including only the largest sample published to date. Identified reports were cross-referenced for inclusiveness. Fig. 1 shows that a total of 199 research papers were identified; 149 were original articles. Of these, 26 studies met inclusion criteria (see Fig. 1 for the breakdown of the selection process), and are summarised in Tables 1 and 2 and discussed below in detail.

3. Results

3.1. Relationship between hair cortisol and stress-related conditions

Fourteen papers were found investigating cortisol in non-pathological stress-related conditions. The details are shown in

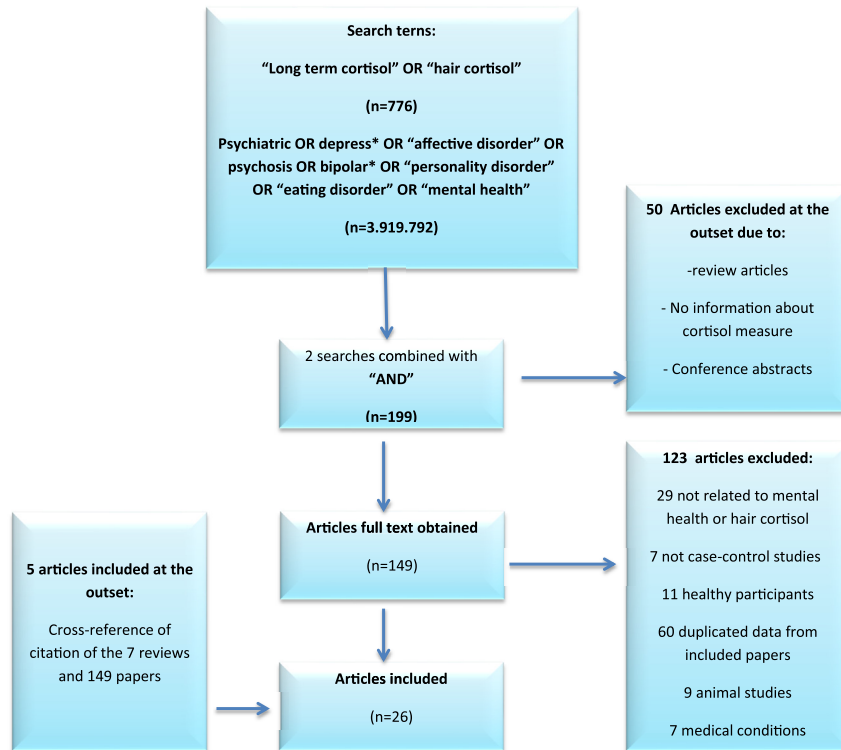


Fig. 1. Flow chart of selection process for inclusion of studies.

Table 1. None of the included studies compared hair with any other samples i.e. saliva, blood or urine. Although these studies used different analytical techniques to extract cortisol, they generally obtained values of similar magnitude. Stressful conditions were found to increase the level of HCC even in those studies in which no association with psychological scales was found (Karlén et al., 2011; Skoluda et al., 2012). Table 1 shows that low cortisol levels were found in one study only (Grunau et al., 2013). In this study procedural pain-induced stress in the neonatal period in preterm children (≤ 32 weeks gestation) was associated with low cortisol in hair at age 7.

3.2. Relationship between hair cortisol and psychiatric illnesses

Twelve original articles were found on this topic (see Table 2). Four investigated major depressive disorder (MDD) (Dettenborn et al., 2010; Hinkelmann et al., 2013; Dowlati et al., 2010; Wei et al., 2015), one bipolar disorder (BD) (Manenschijn et al., 2012), one generalised anxiety disorder (GAD) (Stedte et al., 2011), three posttraumatic stress disorder (PTSD) (Stedte, Kolassa et al., 2011; Stedte et al., 2013; Luo et al., 2012), and three substance misuse disorders (Stalder et al., 2010; Parrott et al., 2014; Grassi-Oliveira et al., 2012). Details of the studies are provided below.

3.3. Major depressive disorder (MDD)

Four articles have investigated HCC in patients with MDD. The first study (Dowlati et al., 2010) measured cortisol concentration in the last three months in MDD patients with coronary artery disease (CAD) after a period of rehabilitation. Patients with MDD and CAD did not have significantly different HCC in comparison to healthy controls. After adjusting for several factors, HCC was not associated with the severity of depression. This study has an important

limitation in that CAD is known to increase cortisol levels (Troxler et al., 1977), potentially counteracting any low cortisol levels due to depression.

The second study (Dettenborn et al., 2012a,b) measured HCC in medicated, moderately depressed patients without serious medical conditions. In this report subjects with MDD had significantly higher HCC than controls. Clinical variables such as severity of the episode, number of past episodes, and length of current episode did not correlate with HCC.

A very recent study by Wei et al. (2015) measured HCC before and during a depressed episode in a group of medication free individuals experiencing their first episode of depression or a recurrence. The authors observed higher hair cortisol level only in the currently depressed first-episode patients group compared with healthy controls and recurrent patients. No significant difference was observed between recurrent patients irrespective of mental state and healthy controls.

In the fourth study Hinkelmann et al. (2013) assessed cortisol concentration in hair and saliva specimens in MDD patients and healthy controls whilst evaluating childhood trauma history. Although the effect of depressive symptoms on HCC was non-significant, the sub-sample of subjects with history of childhood trauma showed a significantly lower HCC and salivary cortisol than those subjects without childhood trauma. A possible interpretation is that early adverse experiences affect the HPA axis irrespective of diagnostic status (Meinlschmidt and Heim, 2005; Klaassens et al., 2012). Some stressors occurring early in life might produce a lasting effect on the stress response, which appears independent of current psychopathology (Hinkelmann et al., 2013).

In conclusion, the few studies which have measured hair cortisol in depression show a more complex picture than the previously reported higher rates of cortisol secretion in MDD (Nemeroff and Vale, 2005) obtained by measuring non chronic levels of this

Table 1
Overview of articles addressing relationship between hair cortisol and stress.

Authors	Type of stress	Sample	Method	Result (mean) (cortisol expressed as pg/mg hair)
(Skoluda et al., 2012)	Physical stress	319 amateur athletes (58.9% female, mean age: 38.3 years) 76 controls (82.9% female, mean age: 38.6 years)	A: one or more 3 cm hair segments B: isopropanol C: ball mill D: methanol E: CLIA	Endurance athletes (18.2) had higher hair cortisol than controls (12.4)
(Manenschijn et al., 2011a)	Shift work	33 shift workers (0% female, median age: 41 years) 89 day workers (0% female, median age: 33 years)	A: first 3 cm B: ? C: ? D: methanol E: ELISA	Shift workers (47.3) had significantly higher hair cortisol than day workers (29.7) Sub-analysis: shift workers <40 yr (48.5) had higher hair cortisol than day workers <40 yr (26.4) Sub-analysis: No difference between shift workers >40 yr and day workers >40 yr
(Dettenborn et al., 2010)	Unemployment	31 unemployed (96.8% female, mean age: 36.7 years) 28 employed (57.1% female, mean age: 32.6 years)	A: first 3 cm B: ? isopropanol C: ball mill D: methanol E: CLIA	Unemployed individuals had higher hair cortisol (23.0) than employed individuals (15.0) (NB these values were extracted from a graph)
(Van Uum et al., 2008)	Chronic pain	14 pain patients (60% female, mean age: 43 years) 39 controls (51.2% female, mean age: 39 years)	A: first 2 cm B: no C: scissors D: methanol E: ELISA	Pain patients (83.1) had higher hair cortisol than controls (46.1)
(Yamada et al., 2007)	Term, preterm neonates hospitalization	38 hospitalized neonates (? % female, mean gestational age (GA) at birth: 31.8 weeks) 22 hospitalized term infants (? % female, mean GA at birth: 38.5 weeks)	A: 10–50 mg hair B: no C: scissors D: methanol E: ELISA	No difference between term (769.1) and preterm neonates (717.9) Sub-analysis: hospitalized preterm infants (747.66) had higher hair cortisol than healthy term born infants (257.3) Sub-analysis: term infants had higher hair cortisol the longer they remained on a ventilator (72.4)
(Grunau et al., 2013)	Neonatal pain-related stress and cortisol levels at school age	91 preterm children (53.8% female, mean age: 7.7 years) 42 term children (64.3% female, mean age: 7.8 years)	A: 2 cm B: isopropanol C: scissors D: methanol E: EIA	Preterm children (173.7) had lower hair cortisol than full-term (239.8)
(Karlén et al., 2011)	Major life stressors	95 students (74.7% female, mean age: 22.1 years)	A: first 3 cm B: ? C: steel ball D: methanol E: RIA	Students with major life events (33.0) had significantly higher hair cortisol than students without major life events (16.3)
(Kalra et al., 2007)	Pregnancy	25 Healthy pregnant women (mean age: 31.5 years)	A: first 1–1.5 cm B: ? C: scissors D: methanol E: ELISA	Maternal hair cortisol levels (48.2) correlated positively and significantly with measures of PSS during the last trimester of pregnancy
(Kramer et al., 2009)	Mothers who delivered Spontaneous preterm birth	31 cases (79.2% range age of 20–34) and 86 controls (83.1% range age of 20–34)	A: first 9 cm B: ? C: ? D: ? E: ?	Hair cortisol in mothers was positively associated with gestational age. Hair cortisol was not significantly associated with pregnancy-related anxiety or any other stress or distress measure. HCC in term control mothers was (69.1) in comparison to cases (62.2). This difference was not significant.
(Henley et al., 2013)	Stress in an Aboriginal community in Canada	40 cases (15 female, mean age: not specified) and 32 controls (gender and mean age years not specified)	A: first 4 cm. B: isopropanol C: scissors D: methanol E: EIA	Median hair cortisol level in the aboriginal community volunteers was 177, significantly higher than the median hair cortisol in the healthy white controls of 116. Hair cortisol correlated positively with gender, smoking status, and self-reported diabetes.
(Groeneveld et al., 2013)	Stress response in children	42 children (54.7% girls, mean age: 4.2 years)	A: first 5 cm, B: no C: scissors D: methanol E: ELISA	Hair analysis was conducted using two 2-cm long segments, reflecting the first 2 months of school attendance (the scalp-near segment) and 2 months prior to school entry. HCC levels were higher (30.02) after school entry than before (27.50), especially for fearful children. Alterations in HCC were not moderated by experience in group day care before school entry.
(Gao et al., 2014)	Earthquake	Cohort A: 20 traumatized (40% female; mean age: 45.0 years) 23 controls (39.1% female; mean age: 41.5 years) Cohort B: 20 traumatized (100% male; mean age: 16.8 years) 29 controls (100% male; mean age: 16.7)	A: first 1–1.5 cm B: methanol C: ball-min D: methanol E: LC-MS/MS	Hair cortisol content (31.9) in cohort A was significantly higher than in controls (7.0). Similarly, hair cortisol content in cohort B at 6 weeks after the earthquake (25.4) was significantly higher than that in its controls (11.7) and remained higher at 22 weeks after

(continued on next page)

Table 1 (continued)

Authors	Type of stress	Sample	Method	Result (mean) (cortisol expressed as pg/mg hair)
(Qi et al., 2014)	Effort-reward imbalance in kindergarten teachers	25 teachers for normally and developmentally disordered children (100% female; mean age?) 14 teachers for normally developing children (100% female, mean age?)	A: first 1 cm B: methanol C: scissors D: methanol E: LC-MS/MS	the earthquake (60 v 45) but not 43 weeks after the earthquake (40 v 35; NB these last two sets of values were estimated from a graph) HCC showed no significant correlation with effort score and reward score, but did show a significantly positive correlation with effort-reward imbalance (values not presented).
(Stalder et al., 2010)	Dementia Caregivers	20 Dementia caregivers (19 female; mean age: 71.2 years) and 20 non-caregivers controls (17 female; mean age: 72.2 years)	A: first 3 cm B: ? C: ball mill D: methanol E: CLIA	Elevated HCC in dementia caregivers (26) compared to non-caregiver controls (20). (NB these values were extracted from a graph). Further, within caregivers, a trend for a positive association of HCC with self-reported caregiving burden ($r = 0.43$, $p = 0.058$) and a positive association with depressiveness ($r = 0.48$, $p = 0.045$) were observed

A: length hair B: washing hair C: cutting (scissors) or pulverization (ball mill) D: Extraction method E: measuring cortisol mg: milligrams cm: centimetre pg: picograms. Abbreviations: CLIA - chemiluminescence immunoassay; EIA - enzyme immunoassay; ELISA - enzyme-linked immunosorbent assay; LC-MS/MS - liquid chromatography-mass spectrometry; RIA - radioimmunoassay.

hormone.

3.4. Bipolar disorder (BD)

Manenschijn et al. (2012) studied salivary cortisol as well as HCC in a population of BD in-patients (type I, type II and not otherwise specified) at different phases of the illness. There were no significant differences between bipolar patients and controls in terms of cortisol levels in saliva or hair. However, they did find significantly elevated HCC in the group of patients who had experienced their first episode of depression or mania after 30 years of age compared with those presenting with depression or mania before age 30 for the first time. This difference was not found in salivary cortisol levels. Significantly lower HCC was found in patients with comorbid panic disorder, which represented 14% of the sample. Furthermore, they found elevated HCC in the group of BD patients with other psychiatric comorbidities (28% of the sample) compared to the BD patients without psychiatric co-morbidities (58% of the sample). However, when patients with co-morbid panic and other disorders were combined, the difference was not statistically significant. This suggests that different comorbid disorders might affect basal cortisol levels in different directions. This study supports the importance of identifying the contribution of comorbidities, especially those frequently occurring in BD (e.g. borderline personality disorder) when cortisol levels are measured.

3.5. Generalised anxiety disorder (GAD)

Steutde et al. (2011b) found that patients with GAD had significantly lower HCC than controls suggestive of hypocortisolaemia in this condition. Salivary analysis, however, revealed that the two groups showed comparable two-day diurnal salivary cortisol profiles. The authors explain the lack of difference in salivary cortisol between the groups as a reflection of a surge of anxiety levels in patients caused by the sampling experience. In this study, using HCC provided a comparative advantage to salivary measurements, being a reflection of chronic levels not affected by acute cortisol variations. The authors concluded that GAD subjects have a lower cortisol baseline that can be affected by acute changes in anxiety levels associated with sampling. Despite this, this study did not measure saliva cortisol reactivity e.g. cortisol awakening response (CAR) or levels following neuropsychological tasks, which would have specifically addressed this issue. A relatively small sample size ($n = 30$), who were almost exclusively female, and the high levels of

comorbidity (73% major depression) are other potential factors limiting the generalizability of the results.

3.6. Post-traumatic stress disorder (PTSD)

There have been three studies measuring hair cortisol in PTSD patients. In the first study Steutde Kolassa et al. (2011) measured cortisol concentrations in patients with PTSD approximately 1 year after the civil war in Uganda. PTSD patients had significantly higher HCC than traumatised-non-PTSD control subjects (TnoPTSD).

In the second study by Steutde et al. (2013), a larger group of PTSD subjects had experienced a traumatic event approximately 5 years earlier. Hair cortisol was measured in this group and compared with TnoPTSD subjects and non-traumatised healthy controls. PTSD patients and TnoPTSD subjects had respectively 59% and 51% lower HCC than non-traumatised individuals. Of interest, such a difference between groups was not found in saliva cortisol measurements. In addition, the study pointed towards a correlation between lower HCC and larger number and frequency of traumatic experiences and a longer interval since traumatization (regardless of the presence of PTSD).

The third study was carried out by Luo et al. (2012) with subjects who had experienced an earthquake in China. Authors collected hair strands from females with PTSD, TnoPTSD and healthy controls. Their results showed that HCC among PTSD and TnoPTSD subjects was similarly elevated compared to controls in the segment of hair corresponding to the time just after the earthquake, suggesting that cortisol levels increase equally, regardless of diagnosis, in response to stress. This reflects the idea that irrespective of any subsequent development of pathology, in the first stage of stress response (equivalent to the alarm reaction according to Selye) the HPA axis behaves similarly in terms of cortisol production (Selye, 1946). However, the PTSD subjects showed significantly lower HCC compared to TnoPTSD subjects in the segments pertaining to 3 and 6 months after the disaster. This suggests that traumatised people without PTSD utilise their own personal resilience to overcome the traumatic event whilst those with PTSD enter the 'exhaustion stage'. This finding of hypocortisolaemia in PTSD using a HCC sample generally concurs with studies that have used other sampling techniques (e.g. Yehuda et al., 1996, 24-h urine samples collection and Rohleder et al., 2004, 4 saliva samples on 2 consecutive days). Furthermore Ehring et al. (2008) found that low salivary cortisol values immediately after a trauma predicted PTSD symptoms 6 months later. However, some other similar studies

Table 2

Overview of articles addressing hair cortisol and mental illness.

Authors	Topic	Sample	Method	Comparison with conventional method	Result (mean) (cortisol per pg/mg hair)
(Steutde et al., 2013)	PTSD	25 PTSD patients (96% female, median age: 36.8 years) 25 traumatized no PTSD (92% female, median age: 41.7 years) 28 non-traumatized controls (89.3% female, median age: 37.6 years)	A: first 2 segments of 3 cm each B: isopropanol C: scissors D: methanol E: LC-MS/MS	Yes. Saliva. 3 saliva measures (on awakening, +30 min, and at bedtime). There is no information on when these saliva measures were taken in relation to the hair sampling	PTSD patients and traumatized control subjects exhibited 59% (6.4) and 51% (7.8) lower HCC in both segments of the previous 6 months than non-traumatized control subjects (15.7), respectively. The overall pattern of HCC associations was not reflected in short-term salivary cortisol findings.
(Steutde, et al., 2011a)	PTSD	10 PTSD patients (60% female, mean age: 19.2 years) 17 traumatized controls (35.3% female, mean age: 20.1 years)	A: first 3 cm hair B: ? C: ball mill D: ? E: CLIA	No	PTSD patients had significantly higher hair cortisol (37.4) than traumatized controls (26.3)
(Luo et al., 2012)	PTSD	32 PTSD patients (100% female, mean age: 13.8 years) 32 traumatized non-PTSD controls (100% female, mean age: 13.8 years) 20 controls (100% female, mean age: 14.4 years)	A: first 12 cm hair (4 segments of 3 cm each) B: isopropanol C: ball mill D: methanol E: ECL immunoassay	No	Significant interaction between group and time strand 4 (baseline): no difference between groups. PTSD (2.0) traumatized non-PTSD (2.0) Controls (2.0)* Strand 3 (2 month before and 1 month after earthquake): PTSD patients and traumatised non-PTSD had higher hair cortisol than controls PTSD (4) traumatized non-PTSD (4.5) Controls (3.0)* Strand 2 (2–4 months after earthquake): non-PTSD patients had higher hair cortisol than PTSD patients who had higher hair cortisol than controls. PTSD (5.2) traumatized non-PTSD (6.5) Controls (4.5)* Strand 1 (5–7 months after earthquake): non-PTSD patients had higher hair cortisol than PTSD patients, hair cortisol in controls was in between PTSD and non-PTSD patients and not significantly different from both patients groups PTSD (5.2) traumatized non-PTSD (6.2) Controls (5.7)* *All these values were extracted from a graph Anxiety patients had significantly lower HCC (11.3) than controls (21.2) in both segments.
(Steutde, et al., 2011b)	GAD	15 GAD patients (86.7% female, mean age: 36.4 years) 15 controls (86.7% female, mean age: 35.6 years)	A: two segments of hair of 3 cm each B: isopropanol C: ball mill D: methanol E: CLIA	Yes. Saliva. Participants collected six saliva samples (on awakening, +30 min, 12:00, 16:00, 20:00 h and at bedtime) on two consecutive weekdays. These days were not specified	
(Dowlati et al., 2010)	MDD + CAD (coronary artery disease)	34 depressed patients (35.3% female, mean age: 61.6 years) 87 controls (19.5% female, mean age: 65.7 years)	A: first 3 cm hair B: no C: scissors D: methanol E: ELISA	No	No difference between depressed patients (4.9) and non-depressed patients (5.0)
(Dettenborn et al., 2010)	MDD	23 depressed patients (65.2% female, mean age: 41.6 years) 64 controls (73.4% female, mean age: 39.9 years)	A: two segments of hair of 3 cm each one B: ? C: ball mill D: ? E: CLIA	No	Depressed patients had higher hair cortisol (24.3) than controls (16.0) in both segments. The severity of the episode, number of past episodes, or length of current episode did not correlate with HCC All these values were extracted from a graph
(Hinkelmann et al., 2013)	MDD and childhood trauma	43 MDD patients (62.7% female, median age: 41.7 years) 41 controls (63.4% female, median age: 41.2 years)	A: first 3 cm hair B: ? C: scissors D: ? E: RIA	Yes. Saliva. Salivary cortisol was collected on 2 consecutive days at awaking and at 12:00 pm, 4:00 pm and 10 pm. There is not information on when these saliva samples were taken.	Results revealed that subjects with history of childhood trauma had significantly lower (3.7 pg/mg) HCC compared with 84 subjects without childhood trauma (5.6 pg/mg) HCC. (These values were estimated from a graph). In contrast, the main effect of diagnosis and the diagnosis × trauma interaction was not significant Before disease episode, no significant differences were observed among healthy controls (13.53 pmol/mg),
(Wei et al., 2015)	MDD	35 MDD patients; 22 (63%) first episode (100% female, mean age:	A: first 1 cm hair and the 3rd or 4th cm from the	No	

Table 2 (continued)

Authors	Topic	Sample	Method	Comparison with conventional method	Result (mean) (cortisol per pg/mg hair)
(Manenschiijn et al., 2012)	Bipolar disorder	35.5 years) 13 (37%) recurrent episode (100% female, mean age: 39.92) 30 controls (? % female, mean age: 37.07)	scalp depending on whether the current episode started 1 or 2 months ago and the first 1 cm in controls B: yes C: ball mill D: methanol E: ECL immunoassay	In 90 patients with BD saliva samples were also collected on two consecutive evenings at 2200 h. There is not information on when these saliva measures were taken.	first-episodic patients (10.01 pmol/mg) and recurrent patients (11.20 pmol/mg). In disease episode, the hair cortisol level in first-episodic patients (19.24 pmol/mg) was significantly higher than that in healthy controls (13.53 pmol/mg) or recurrent patients (14.35 pmol/mg), while no significant difference was observed between recurrent patients and healthy controls.
(Stalder et al., 2010)	Alcoholism	100 bipolar patients (62% female, median age: 52 years) 195 controls (52.8% female, median age: 32 years)	A: first 3 cm hair B: no C: scissors D: methanol E: ELISA	No	No difference between patients (31.8) and controls (28.1) Subanalysis: No effect of disease state Subanalysis: patients with age of onset >30 yr had higher hair cortisol than patients with age of onset <30 yr (comparable to controls) Subanalysis: patients with comorbid panic disorder (22.1) had lower hair cortisol than patients without panic disorder (34.6) Subanalysis: patients with other comorbidities (44.8) had higher hair cortisol than patients without other comorbidities (31.4)
(Parrott et al., 2014)	Heavy MDMA users	23 alcoholics in acute withdrawal (8.7% female, mean age: 44.3 years) 25 abstinent alcoholics (24% female, mean age: 45.6 years) 21 controls (11.2% female, mean age: 43.6 years)	A: first 3 cm hair B: ? C: ball mill D: ? E: CLIA	No	Patients in acute withdrawal (51.9) had higher hair cortisol than abstinent patients (13.9) or controls (16.3) Note: the hair segment of the withdrawal patients reflect the active drinking phase
(Grassi-Oliveira et al., 2012)	Crack cocaine users	27 recent light users (33.3% female, mean age: 21.2 years) 23 heavy users (65.2% female, mean age: 21.5 years), 51 controls (47.1% female, mean age: 21.2 years)	A: first 3 cm hair B: ? C: scissors D: ? E: CLIA	No	Hair cortisol levels were observed to be significantly higher in heavy MDMA users (mean = 55.0), compared to recent light MDMA users (19.4), and to non-users (13.8).
		23 crack cocaine-dependent inpatients (100% female, mean age: 29.6 years)	A: 1 cm hair B: ? C: scissors D: methanol E: ELISA	No	HCC decreases after 30 days prior to admission (24.4), 60 days prior to admission (21.0) and 90 days (15.9).

A: length hair, B: washing hair, C: cutting (Scissors) or pulverization (Ball mill), D: Extraction method, E: measuring cortisol, mg: milligrams, cm: centimetre, pg: picograms. Abbreviations: CLIA - chemiluminescence immunoassay; ECL - electrochemiluminescence; EIA - enzyme immunoassay; ELISA - enzyme-linked immunosorbent assay; LC-MS/MS - liquid chromatography-mass spectrometry; RIA - radioimmunoassay.

reported hypercortisolaemia in PTSD (e.g. [Maes et al., 1998](#) and [Rasmusson et al., 2001](#) by using 24-h urine samples collection). Aside from sampling methodologies, confounding factors included physical and psychiatric comorbidity, and the differing nature of the stressors studied. Temporality also appears to be a particularly important confounder in this type of PTSD research as some studies were carried out months after the traumatic event while others were executed years later.

3.7. Alcohol and substance misuse disorders

A study by [Stalder et al. \(2010\)](#) found that acute withdrawal was associated with significantly higher HCC compared with levels measured in both long-term abstinent patients and healthy controls, the latter two groups having similar levels. These results support the notion that hair cortisol specimens might be used to monitor recovery from alcohol misuse disorders. Recently, [Parrott et al. \(2014\)](#) found significantly higher HCC in heavy MDMA users in comparison to light users and controls. In the third study, undertaken in female inpatients receiving treatment for crack cocaine dependence, [Grassi-Oliveira et al. \(2012\)](#) found a positive association between measures of HCC and number of stressful events. As the findings were correlational in nature conclusions are open to methodological limitations (see [Table 2](#)).

4. Discussion

There is increasing evidence that hair can be used to measure chronic levels of cortisol in many disorders including conditions of stress and psychiatric disorders. It is, however, noticeable that the replicability of this methodology, especially when applied to psychiatric disorders, is at present questionable. The studies described in this review clearly demonstrate a high rate of variability among research reports. Furthermore, in a range of psychiatric disorders, there is discordance between measurements in hair and saliva so that significant discrepancies are found in the pattern of cortisol secretion obtained from these two measures.

4.1. Stress response in stress conditions and disorders

The most striking finding that emerges from this review is that hair specimen analysis has been shown to be a useful tool to detect high cortisol levels in healthy individuals under stress ([Table 1](#)). This line of research shows that the presence of hypercortisolaemia does not necessarily reflect poor health ([Gerber et al., 2012](#)). Among all the studies which investigated HCC in a stress condition, only one study by [Grunau et al. \(2013\)](#) found low HCC. This study differed from the others as it investigated a sample of neonates who experienced high level of stress. However, hair samples were taken years after the stressful situation – at the age of 7 – which does not directly contradict results from the other studies. In fact, [Palmer et al. \(2013\)](#) confirmed that children also show high HCC when the stressful situation is measured at the time stress is experienced. Therefore, it seems that stressors might have not just an acute effect but also long-term effects on basal levels of HCC if they occurred very early in life. In agreement with [Grunau et al. \(2013\)](#), this has also been confirmed by [Hinkelmann et al. \(2013\)](#) who found low HCC in adult subjects with a history of childhood trauma, independent of current psychopathology.

Another factor relevant to HCC and stress response is the cognitive appraisal of the stressor. [Tachè and Selye \(1985\)](#) initially described the different quality of 'positive' (eustress) and 'negative' (distress) stressors. [Staufenbiel and Koenders \(2014\)](#) further demonstrated an association between number of stressors and high HCC driven by the presence of negative stressors.

Studies on stress disorders, PTSD especially, have provided good quality evidence on the relationship between stress and psychiatric disorders related to stress. Following a traumatic event, levels of cortisol tend to increase. In PTSD an abnormal stress response is associated with an initially potentiated response followed by chronic cortisol levels below the baseline often irrespective of whether the stressor was acute ([Luo et al., 2012](#)) or chronic ([Steudte et al., 2013](#)). This type of response seen in PTSD emphasises the importance of the effect of the stress reaction in the individual rather than the stressor itself ([Selye, 1974](#)). It follows that the term chronic stress in this context might appear vague, arbitrary and confusing. In this context, the alternative use of the term 'normal' or 'abnormal stress response' would seem a more appropriate descriptive terminology. In PTSD studies, variability is introduced by the interval between the occurrence of the traumatic event and the time of hair sampling. Basal cortisol may be increased when the traumatic event occurred in more recent past, compared to more prolonged intervals. [Steudte, Kolassa et al. \(2011\)](#), for example, found hypercortisolaemia in a group of war survivors when cortisol was measured one year after the conclusion of the conflict. [Steudte et al. \(2013\)](#), however, found hypocortisolaemia when studying a sample of individuals exposed to a traumatic event 5 years earlier.

In contrast to the views of [Staufenbiel et al. \(2012\)](#), the present authors argue that, based on the results of PTSD studies carried out with hair cortisol, describing the stress response in terms such as 'reaction', 'recovery' and 'adaptation' might be overly simplistic. These studies in fact suggest that the 'adaptation stage' is not always the ultimate conclusion of every stress response. It seems more appropriate to view the 'exhaustion stage' as an alternative to the adaptation stage. This alternative term was used by [Selye \(1946\)](#) in his classic work and more recently by [Goldstein and Kopin \(2007\)](#).

Similarly, the view that dysregulation of the HPA axis is a chronic phenomenon referred to as 'allostatic load' ([Staufenbiel et al., 2012](#)) may not necessarily be the case in stress related disorders such as PTSD. This is because central to the 'allostatic load' index is the notion of hyperactivity of the HPA axis. Findings from this work in fact suggest that chronic dysregulations in stress related disorders such as PTSD may well present with hypoactivity of the HPA axis. It follows that the nature of the link between cortisol levels and stress related disorders requires further investigation preferably with newly developed indexes of HPA function to take into account the possibility of axis hypoactivity.

4.2. Measuring stress using hair analysis

An important issue worth considering is that of the optimal way to measure stress. In relation to psychological questionnaires, the Perceived Stress Scale (PSS) ([Cohen et al., 1983](#)) is a widely used and validated self-report questionnaire that quantifies stress perceptions over a set period of time (e.g. the past month). This scale has been used in conjunction with biological markers. For instance, [Faresjö and Jullander \(2014\)](#) found that middle-age women reporting high scores on the PSS had high HCC. In a recent study, [Wells et al. \(2014\)](#) found a possible inverse U relationship between PSS scores and HCC, suggesting that the effect of stressor intensity may play a crucial role in the development of low HCC that the previous PTSD studies in hair have shown.

Despite the previous positive examples of PSS measuring stress, it seems that the sensitivity of this questionnaire to detect subjects under stress conditions has not been as accurate as hair specimens finding high levels of cortisol in the same group of subjects. For instance, stress conditions such as spontaneous pre-term labour in pregnant women ([Kramer et al., 2009](#)) or high intensity sport in endurance athletes ([Skoluda et al., 2012](#)) showed high HCC but no differences in PSS scores in comparison with healthy controls. This

may indicate that not every physiologic stress response is associated with a cognitive stress response, a concept possible to extend to the individual significance of life events. [Karlén et al. \(2011\)](#) for example found that HCC was significantly related to the presence of negative life events, but weakly negatively correlated with the PSS.

Hair cortisol measurement may prove to be superior to other biological specimens in measuring stress in acute settings. Acute salivary cortisol reactivity, sweat cortisol levels, sweating rate or diurnal variation are less likely to affect HCC measurements in comparison to acute cortisol specimens. Cortisol diurnal variation was shown to be the least likely to affect HCC measurements ([Grass et al., 2015](#)). Furthermore, [Dettenborn et al. \(2012b\)](#) demonstrated that tobacco, another confounding factor in saliva specimens, does not interfere with cortisol levels in hair specimens.

4.3. A novel detection method of stress response?

In view of the limited validity and sensitivity of methods and rating scales in reliably detecting sustained stress responses, hair cortisol measurement might be considered a potentially viable approach to identify responses to stress persisting beyond the initial trigger leading to 'resistance' or 'exhaustion'. Selye first noted that not every trigger causing an alarm reaction in an individual can be considered a 'stressful event' ([Selye, 1974](#)). It follows that a reliable chronic measurement of cortisol might prove more effective in profiling cortisol concentrations to discriminate a stress response. For instance, [Skoluda et al. \(2012\)](#) demonstrated that only endurance athletes exhibited significant alteration in hair cortisol versus healthy controls, a change not seen in individuals who underwent moderate, non-competitive levels of exercise. This reflected the level of stress endurance athletes were experiencing. Further longitudinal work is required to validate the role of hair cortisol in detecting stress responses.

4.4. Hair cortisol in psychiatric disorders

The relationship between HCC and psychiatric disorders is not as close as that found with stress conditions; findings tend to diverge and are often conflicting. The overall number of studies available is also limited. By using depression and anxiety disorders as an example, study outcomes differ according to the characteristics of the samples investigated. For instance, [Dowlati et al. \(2010\)](#) included in their study participants with coronary artery disease, a condition known to alter cortisol concentration ([Troxler et al., 1977](#)). Two of the reviewed reports ([Wei et al., 2015](#) and [Dettenborn et al., 2012a,b](#)) showed higher HCC in depressed individuals compared with controls; this is in contrast with the other two studies ([Hinkelmann et al., 2013](#); [Dowlati et al., 2010](#)). [Manenschijn et al. \(2012\)](#) did find differences in HCC in BD, but only when comparing samples with and without comorbidities. Their sample was composed of people with BD in different phases of the illness and with a high percentage of comorbidities, mainly personality and panic disorders. This suggests that comorbid psychiatric and physical disorders need to be accounted for when conducting these kinds of studies.

In many studies in which cortisol was measured using hair as well as saliva samples, a discrepancy between the pattern of cortisol secretion obtained from these two measures has been observed. This is likely due to the fact that the HPA axis is a highly reactive system. A clear example is the GAD study where saliva samples found normocortisolaemia whilst hair samples found hypercortisolaemia. In their study on BD patients, [Manenschijn et al. \(2012\)](#) found that, in contrast to the decreased levels of cortisol found in hair, co-morbid panic disorder was associated with a trend towards increased salivary cortisol levels. This again

shows the variability of saliva samples and how they may be unsuited as a measure of basal long-term cortisol secretion. This also suggests that anxiety disorders have low HCC.

As the number of studies per diagnosis was limited, preferably longitudinal studies are needed to further unravel the clinical usefulness of measuring HCC in patient populations.

4.5. Methodological issues in hair analysis

Variation in the procedures used in the manipulation of hair specimens and their analysis is one of the obstacles to the standardisation of the technique to maximise generalisation and comparability of findings. The use of different immunoassays and liquid chromatography techniques (e.g. LC-MS and LC-MS/MS methods) across different laboratories is an example. It is however worth noting that in a recent paper, [Russell \(2015\)](#) demonstrated that HCC determined by four immunoassay methods were highly and positively inter-correlated in all comparisons of individual laboratories.

Another unknown factor is whether the secretion of sweat in the scalp affects hair cortisol measurements. A recent study by [Grass et al. \(2015\)](#) demonstrated in two experiments that this effect can be minimised by measuring cortisol from the hair shaft where it is least likely to be altered. To further remove contamination from sources of cortisol like sebaceous and sweat glands, some researchers have also used hair specimens washed with methanol before cortisol extraction.

That other factors such as diurnal variability, cortisol reactivity, sweat or tobacco have no effect on cortisol values measured in hair makes this specimen a reliable candidate to determine accurately and with validity any changes in chronic cortisol values that may result from, or contribute to psychiatric illness. This is a potentially important step in increasing the diagnostic specificity and categorisation of symptom cluster subtypes. However, the few studies undertaken in psychiatric illnesses to date have not allowed definitive conclusions in relation to this so far.

Another advantage of hair over other specimens is the ability to allow tracking of cortisol production around specific events that occurred in the past, due to the retrospective nature of its analysis. If an event – for example treatment initiation or a specific life event – happened one month prior to the hair sampling, only 2 cm of hair from the scalp would be needed to measure any changes in cortisol from the month before to the month after the event. This could also now be extended to other steroid hormones, such as sex hormones and dehydroepiandrosterone (DHEA) ([Yang et al., 1998](#); [Kintz et al., 1999](#)) assays for which have now been developed for hair specimens. These other hormones are also thought to be involved in psychiatric disorders ([McEwen and Alves, 1999](#); [Markopoulou et al., 2010](#)). We suggest that future investigations into the role of these other hormones in stress and mental illness could also be facilitated by hair sample analysis, with similar advantages to those we have described for cortisol.

The length of hair samples used for analysis in these studies is also an important methodological consideration. People from different ethnicities have different hair growth rates; for example, Asian hair is believed to grow at a rate of around 1.3 cm/month instead of the standard measure of 1 cm/month used by [Luo et al. \(2012\)](#). This means that the segments used in this study (and others) may not accurately reflect the presumed time periods they were intending to analyse. Rates of growth have been found to vary from 12.9 to 43.6 $\mu\text{m/day}$ in African populations, from 165 to 506 $\mu\text{m/day}$ in Caucasian populations and from 244 to 611 $\mu\text{m/day}$ in Asian populations ([Loussouarn et al., 2005](#)).

Variability has also been shown in relation to the methods used to prepare hair prior to cortisol extraction; e.g. hair specimens

could be minced with scissors or pulverised using a ball-mill. Davenport et al. (2006) found 3.5 times more cortisol in hair when it was pulverised compared to un-pulverised hair. This difference was also found by Eser et al. (1997). Although potentially very important, this information is not always reported in studies.

A final factor to mention is the importance of taking into account the washout effect. Cortisol levels in hair decrease over time (Dettenborn et al., 2010). As a result, studies which measure cortisol in very long strands of hair may measure lower levels of cortisol in the segments farthest from the scalp for reasons not necessarily connected to stressor or illness effect. There is, however, no consensus on which segments of hair this effect pertains to and the magnitude of this factor. Thomson et al. (2010) for example found no washout effect even after cortisol was measured over a period of 18 months.

5. Conclusion

Hair cortisol analysis is a new, non-invasive method that is potentially able to provide a long and medium-term retrospective measure of cumulative cortisol secretion in contrast to other specimens such as blood, urine and saliva (Gow et al., 2010). Research to date has found that hair cortisol is reliably affected by environmental stress, and is a useful additional tool to measure such stress over and above current cortisol sampling methods and/or questionnaires. Hair cortisol as a measure of chronic levels obtained in conjunction with acute measures of cortisol secretion could provide a more complete picture as to how cortisol levels vary across the different stages of the stress response. Whilst the use of hair cortisol measurements might contribute to improving the diagnostic validity and/or sub-typing of psychiatric disorders, results to date have been inconclusive and have not found reliable associations between cortisol levels and individual disorders. However, the majority of studies on hair cortisol undertaken in psychiatric disorders have used a cross-sectional, case–control design. Prospective studies with a longitudinal approach might help more effectively disentangle causal relationships between stress, cortisol and psychiatric disorder, and as a long term consequence, facilitate methods to improve outcomes and prognosis for patients.

Conflicts of interest

The authors report no potential or actual conflicts of interest.

Contributors

Dr Herane Vives, Prof. Cleare & Dr. Papadopoulos conceived the design and methodology. Dr Herane Vives conducted the systematic review, as well as primarily writing the manuscript. Dr. Arnone and V de Angel advised on and contributed to systematic review procedures. Dr. Arnone, V de Angel, R Strawbridge, T Wise, Prof. Young and Prof. Cleare supplied further expertise regarding data interpretation. All authors contributed critical revisions and approved the final manuscript.

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