Clinical applications of cortisol measurements in hair

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Abstract

Cortisol measurements in blood, saliva and urine are frequently used to examine the hypothalamus–pituitary–adrenal (HPA) axis in clinical practice and in research. However, cortisol levels are subject to variations due to acute stress, the diurnal rhythm and pulsatile secretion. Cortisol measurements in body fluids are not always a reflection of long-term cortisol exposure. The analysis of cortisol in scalp hair is a relatively novel method to measure cumulative cortisol exposure over months up to years. Over the past years, hair cortisol concentrations (HCC) have been examined in association with a large number of somatic and mental health conditions. HCC can be used to evaluate disturbances of the HPA axis, including Cushing's syndrome, and to evaluate hydrocortisone treatment. Using HCC, retrospective timelines of cortisol exposure can be created which can be of value in diagnosing cyclic hypercortisolism. HCC have also been shown to increase with psychological stressors, including major life events, as well as physical stressors, such as endurance exercise and shift work. Initial studies show that HCC may be increased in depression, but decreased in general anxiety disorder. In posttraumatic stress disorder, changes in HCC seem to be dependent on the type of traumatic experience and the time since traumatization. Increased hair cortisol is consistently linked to obesity, metabolic syndrome and cardiovascular disease. Potentially, HCC could form a future marker for cardiovascular risk stratification, as well as serve as a treatment target.

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Introduction

The glucocorticoid hormone cortisol is produced by the hypothalamus–pituitary–adrenal (HPA) axis, and has effects on virtually all tissues and processes in the human body (1). Biochemical evaluation of cortisol, including dynamic testing, is the cornerstone of diagnosing hypo- and hypercortisolism (2). Besides these obvious endocrine disorders, subtle variations in cortisol exposure

and sensitivity have been extensively studied in relation to a wide range of health and disease characteristics, including aspects of metabolism, inflammation, cardiovascular disease and behavior (1).

Systemic cortisol levels are highly variable, which is due to the diurnal rhythm, acute stress and pulsatile secretion (3). Measurements of cortisol in blood, saliva

Invited Author's profile

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and urine only represent time-point or short-term cortisol exposure, which is limiting the representation of long-term circulating cortisol levels. Furthermore, these tests either rely heavily on patient adherence to collection instructions, or are perceived as invasive (2).

Scalp hair analysis has been used for decades to measure exposure to environmental toxins and drugs. Hair grows at a relatively constant rate of 1 cm/month. Several substances are retained in the hair strands, therefore, hair can serve as a matrix to measure long-term exposure to a wide variety of toxins and hormones. Furthermore, by dividing hair samples in different segments and analyzing them separately, retrospective timelines of exposure can be created (4).

In the past years, several laboratories have expanded hair analysis to measure long-term levels of cortisol and other steroid hormones (5, 6, 7, 8, 9, 10). This has allowed researchers to investigate cortisol exposure over much longer periods of time (months to years) than previously possible with samples of blood, saliva or urine. Consequently, a large number of cross-sectional studies have been conducted and examined the associations between hair cortisol concentrations (HCC) and a wide range of somatic and mental health measures (11, 12). In this review, we will highlight current and potential future applications of hair cortisol measurements, embed this relatively novel method in the context of other available tests, and discuss potential confounders and future directions of HCC measurements.

Hair sample collection and analysis

Scalp hair collection is straightforward and easily performed in an outpatient setting (Fig. 1). In accordance

with guidelines published by the Society of Hair Testing, the hair sample is collected from the posterior vertex (4). Scissors are used to cut a lock of hair as thick as a pencil, as close to the scalp as possible. Depending on the research question, hair segments of one, up to multiple centimeters of length are used for analysis. Samples are weighed, and in most published methods washed. Steroids are extracted in methanol overnight and are then processed further depending on the type of analysis used. The extracted cortisol can be measured using an immunoassay or liquid chromatography tandem–mass spectrometry (LC–MS/MS) (5, 6, 7, 8, 9, 10).

Recently, a direct comparison between hair cortisol analysis methods was published. Four different laboratories divided hair samples of the same persons and measured them using their different methods, comparing four immunoassay methods and two LC–MS/MS methods. Correlations between the different methods were high, with r^2 values ranging between 0.88 and 0.98 (13).

Applications in clinical practice and research

Thus far, HCC have been associated with a wide range of clinical outcomes, the results of which are summarized in Table 1. The published clinical outcomes can be divided in several categories: Cushing's syndrome (CS), hydrocortisone replacement therapy monitoring, cardiometabolic status, psychopathology and either physical or psychological forms of chronic stress. Other factors that can influence HCC, such as sex, age and hair-related factors, are discussed in 'Factors affecting HCC' section.

Figure 1

Overview of hair sample collection, work-up and analysis. ELISA, enzyme-linked immunosorbent assay LC–MS/MS, liquid chromatography–tandem mass spectrometry.

Table 1 Clinical and situational factors associated with higher or lower hair cortisol levels.

	Increased hair cortisol	Decreased hair cortisol
Somatic health factors	Cushing's syndrome Hydrocortisone use Obesity Metabolic syndrome Diabetes mellitus Cardiovascular disease Heart failure severity Recent myocardial infarction	Childhood asthma with inhalation glucocorticoids
Chronic and acute stressors	Intensive aerobic exercise Trauma Life events Unemployment Shift work Severe chronic pain	Traumatic experience
Psychopathology	PTSD ^a Major depressive disorder Bipolar disorder, late onset	PTSD ^a Generalized anxiety disorder Panic disorder

^aPosttraumatic stress disorder (PTSD) has been associated with both increased and decreased hair cortisol concentrations (depending on the type of traumatic event, characteristics of the patient sample examined, and the timespan between the trauma and assessment), when compared to controls.

Cushing's syndrome

We and others have previously reported increased HCC in patients with endogenous CS (14, 15, 16). In a sample of 14 CS patients and 96 healthy controls, we found a sensitivity of 86% and a specificity of 98% for CS, which is comparable to the diagnostic accuracy of urinary free cortisol (UFC), late night salivary cortisol (LNSC), and the low-dose dexamethasone suppression test (DST) (14). Since our study only included 14 patients with CS and was not designed to compare HCC with other tests, future studies have to be awaited to in order to determine the value of HCC in diagnosis and surveillance for recurrence of CS. Cortisol secretion is known to be highly variable in CS, with a majority of patients showing at least one normal UFC or LNSC in repeated testing (17). By providing information about cortisol exposure over a much longer timeframe, measurement of HCC may help to overcome this issue. Furthermore, unlike current first-line screening tests for CS, hair sample collection is not limited by patient adherence to collection instructions (as with UFC or LNSC) or to medication intake (as with the low-dose DST).

One of the main advantages of hair measurements is the possibility to create retrospective timelines of cortisol exposure, months up to years back in time, depending of the length of the hairs. Furthermore, depending on the length of the hair, HCC may be used to indicate the time of onset of the disease. We and others have described cases

of CS in which the timelines of HCC corresponded with disease course (14, 15, 16), and showed that creating timelines can be especially helpful in cases of suspected cyclic CS. In cyclic CS, duration of episodes of hypercortisolism and normocortisolism are unpredictable, varying from several days to months or years. Cyclic CS can therefore be very challenging to diagnose, because cortisol production may appear normal in biochemical evaluation (18). By expanding the time window in which cortisol is measured, and the addition of retrospective timelines, which can be individualized based on the clinical symptoms of the patient, HCC have the potential to improve the detection of cyclic CS cases considerably.

Hydrocortisone replacement therapy monitoring

Hydrocortisone prescribed for adrenal insufficiency (AI) is chemically identical to cortisol. Therefore HCC may be used to assess long-term systemic levels of administered hydrocortisone in AI patients. Several published studies have investigated HCC in patients treated with hydrocortisone for AI. The studies included 132 adults with AI, 93 adults with AI, 54 children with AI and 15 adults who were treated with the adrenolytic drug mitotane for adrenocortical carcinoma (19, 20, 21, 22). All studies reported higher mean HCC in hydrocortisone-treated patients compared to healthy individuals or non-AI controls. In the two studies performed in adult AI patients,

HCC correlated significantly with hydrocortisone dose (19, 20), while in the other two studies, HCC correlated significantly with BMI but not with hydrocortisone dose (21, 22). In the adrenocortical carcinoma patients, this can be explained by mitotane treatment, which alters the metabolism of hydrocortisone. These results indicate that HCC may be used to identify overtreatment with hydrocortisone. Combining HCC with clinical symptoms of hydrocortisone overtreatment may therefore improve refinement of individualized hydrocortisone substitution therapy. However, glucocorticoid action is affected by a large number of factors. These include intra-individual differences in hydrocortisone absorption and breakdown which are likely reflected in hair levels, but also differences in glucocorticoid sensitivity which alter cortisol effects at the tissue level (1).

Next to hydrocortisone replacement therapy, other glucocorticoids are commonly used to treat a wide variety of diseases (e.g. inflammatory and autoimmune diseases, malignancies). Glucocorticoid use may suppress the HPA axis, but since hydrocortisone is identical to cortisol, HCC cannot be used to distinguish between exogenous and endogenous cortisol. However, other synthetic glucocorticoids may be distinguished from cortisol by HCC measurements. One recent study found lower HCC in children who used inhaled glucocorticoids for asthma, providing preliminary evidence that HCC might be used to detect HPA axis suppression due to exogenous glucocorticoid use (23).

Cardiometabolic status

Excessive glucocorticoid exposure is associated with increases in cardiometabolic risk factors such as obesity, dyslipidemia, glucose intolerance and hypertension (24). However, these observations come from patients with an overt excess of glucocorticoids due to glucocorticoid therapy or endogenous hypercortisolism. Studies using cortisol levels derived from body fluids could not fully unravel the relationship between variations in cortisol exposure within the physiologic range and cardiovascular risk (24). Cross-sectional studies using hair analysis have shown that higher HCC are associated with an adverse cardiovascular risk profile. We have found that in a population of 283 elderly, individuals in the highest quartile of HCC were 2.7 times more likely to suffer from cardiovascular disease, whereas no relationship with non-cardiovascular chronic disease (e.g. cancer and obstructive lung disease) was found. Furthermore, HCC were positively associated with presence of type 2 diabetes

mellitus (25), a finding which was confirmed in another population-based elderly cohort (26). HCC have been found to be increased in patients with a recent myocardial infarction (27). In a large cohort of 1258 middle-aged aviation workers, Stalder *et al.* (28) found higher hair cortisol and cortisone levels associated with increased presence of the metabolic syndrome, showing that the relationship between HCC and adverse cardiometabolic status is already present earlier in life.

The association between cortisol levels and obesity has been a subject of research for a long time. Results from past studies are conflicting. Urinary cortisol has been reported increased in obese individuals compared to lean individuals, but this increase is generally not reflected in serum or salivary cortisol levels (24). We found that in both adult and pediatric obese patients, HCC are increased compared to non-obese healthy controls (29, 30). Furthermore, HCC positively correlates with BMI in multiple other studies (21, 28, 31, 32, 33, 34). Together, these results strongly suggest that increased long-term cortisol exposure is associated with increased adiposity. An increased long-term cortisol exposure may possibly be responsible for part of the increased cardiovascular disease risk in obese individuals. However, no causal inferences can be drawn from these studies. Long-term cortisol exposure may not only be important in developing an increased cardiovascular disease risk profile, but may also have modulating effects in cardiac disease. This is illustrated by the finding that higher HCC have been associated with worse functional status in heart failure (35), and with worse subjective physical health in structural heart disease (32).

Psychopathology

Cortisol is thought to play an important role in psychopathology, in particular in mood and anxiety disorders. Depressed individuals have been found to take more time to return to baseline cortisol levels in response to a psychological stressor compared to non-depressed controls (36). Furthermore, patients with current and remitted depression appear to have an increased cortisol awakening response (37). An increased HPA axis reactivity in patients with depression may be caused by a relative resistance to cortisol, as evidenced by a blunted response to the DST in depressive patients (38). One relatively small study, involving 23 patients with major depressive disorder and 64 matched controls, found an increase in HCC in depressed patients using antidepressant medication (39). In patients with bipolar disorder we also identified a subgroup with an older age of onset having increased

HCC (40). These results indicate that an altered HPA axis activity in mood disorders may result in increased long-term cumulative cortisol levels.

Cortisol is also thought to play a role in anxiety disorders. In a small sample of patients with generalized anxiety disorder (GAD), decreased HCC have been reported. This is in contrast to results obtained from short-term or acutely circulating concentrations, which have been reported to be increased in GAD (41). In a population of patients with bipolar disorder, we have found that those who had a comorbid panic disorder had decreased HCC, while salivary cortisol levels were unaffected by panic disorder in this study (40). These discordances could be explained by the fact that a research setting can form an acute stressor, which could increase acutely circulating cortisol levels, but not affect cortisol levels in scalp hair. Furthermore, these examples demonstrate clearly that hair cortisol, representing long-term cumulative exposure, can offer valuable new information in addition to the time point measurements, which are more suitable to obtain insight in acute changes.

Traumatic experience and posttraumatic stress disorder (PTSD) have also been associated with alterations in HPA axis function. A traumatic experience will result in acutely increased cortisol levels due to an acute stress reaction. Observational studies suggest that after the traumatic event individuals who develop PTSD have lower circulating cortisol levels. However, a recent meta-analysis failed to show an overall difference in cortisol levels in urine, blood or saliva between people with PTSD ($n=828$) and controls ($n=800$) (42). Subgroup analysis in this study revealed lower short-term or time point cortisol levels in female PTSD patients, and in PTSD subjects who were traumatized due to physical or sexual abuse, but not in those who were traumatized due to war. Furthermore, a decrease was only observed in studies in which PTSD subjects were compared to control subjects who were not exposed to trauma, but not when compared to traumatized controls without PTSD (42). This indicates that trauma exposure, rather than the subsequent development of PTSD is responsible for a decrease in cortisol. Findings concerning HCC and PTSD are largely in line with this. Steudte *et al.* (43) reported that both PTSD and non-PTSD traumatized controls both have lower HCC when compared to nontraumatized controls. However, Luo *et al.* described that in adolescent females who experienced an earthquake, HCC was lower in the 32 subjects who developed PTSD, than in 32 persons who did not. This relative decrease was seen in hair segments corresponding to the second through seventh month after

the earthquake, but not before that time (44), indicating that the decrease in cortisol levels only occurs after the traumatic event. Moreover, in another study involving 202 women who survived the same earthquake, HCC levels were not associated with PTSD symptoms 4.5 years after the event (45). Contrary to decreases that are observed in most studies, in 27 traumatized individuals from a civil war area in Uganda, those who developed PTSD had significantly higher HCC (46). In conclusion, the association between long-term cortisol exposure and PTSD appears to be dependent on the type of traumatic event, characteristics of the patient sample examined, and, importantly, the timespan between the trauma and assessment.

Chronic stress

Both physical and psychological stress can induce a biological stress response in the human body, including a hyperactivation of the HPA axis. Much of what is known about HPA axis responses to stressors is derived from measurements of cortisol in saliva. In particular, salivary cortisol is used to investigate changes in the cortisol awakening response, and the response to experimentally induced stress, such as in the Trier Social Stress test (47). As mentioned before, HCC can measure cortisol levels over extended periods of time, and unlike salivary and serum measurements, is not affected by the influence of acute stress or time-of-day at the moment of sampling. Therefore, it is a highly attractive method to investigate the association between real-life stressors and long-term cortisol exposure.

Examples of physical stressors are severe diseases, extreme exercise and starvation. Another common physical, and maybe also partially psychological stressor is disruption of the circadian rhythm (11). We have reported an increase in HCC in shift workers, compared to day workers (31). This is an important finding, because shift work is associated with metabolic syndrome components, and cortisol may be in part responsible for this association. Interestingly, in this study BMI correlated positively with HCC (31). Other examples of physical stress, associated with elevated HCC are endurance exercise (48) and chronic pain (49).

The relationship between experienced psychological stress and the HPA axis is complex. Conflicting results have been reported in the association between perceived stress, and HCC. Both increased (50) and decreased HCC (51) have been associated with higher perceived stress, while other studies have failed to find a relationship

altogether (33, 52). This heterogeneity could be a consequence of the diverse composition of study samples, which range from coronary artery disease patients (52) to college students (51). However, published research supports an association between recently experienced (negative) life events, such as loss of a loved one or an important exam, and increased HCC or hair cortisone in college students (51), elementary school girls (53), patients with bipolar disorder (54) and crack cocaine users (55). Other psychological stressors which have been associated with increased HCC are school entry in children (56), giving care to a relative with dementia (57), promotion prospects (58) and unemployment (59).

Factors affecting HCC

Apart from the clinical states and stressors that we discussed in this review, several other factors can potentially affect HCC. In the largest published studies, HCC increased with age (26, 28). Also, children have been found to have significantly lower HCC than adults (60). Several published studies report higher HCC in males than in females (25, 26, 61), but in other studies no sex difference was observed (9, 28, 33). In two studies carried out in older adults, men had higher hair cortisol, indicating that the sex difference in HCC becomes more pronounced later in life (25, 26). The associations between ethnicity and HCC have largely been unexplored, and need further study.

Multiple studies have confirmed a hair growth rate that closely ~ 10 mm/month (62). Slight differences in hair growth rate between ethnic groups have been reported, with African hair growing more slowly than Caucasian hair, which in turn grows more slowly than East Asian hair (63). Small variations in growth rate are not expected to have a large impact on studies using single measurements of long-term cortisol, but should be considered when creating longer retrospective timelines.

Hair treatments such as hair dyeing, permanent curling or straightening have been reported to decrease HCC (9, 28, 64), although other studies have not found this (6, 33, 48, 52, 60, 65). In the largest published HCC study thus far, in which hair glucocorticoids were measured in 1258 individuals using LC-MS/MS, cortisol and cortisone were lower in hair which was colored, permanently curled or straightened. Furthermore, the amount of cortisone decreased with higher hair washing frequency in this study (28). Another hair-specific limitation is the phenomenon of wash-out, which means that hair cortisol is lower more distally in the

hair, for instance due to wear and tear, subsequent hair washings or exposure to ultraviolet light. We have previously found that HCC was stable in subsequent hair segments in 28 women over a hair length of 18 cm measured using an immunoassay (9), but we did observe a decrease in cortisol levels along the hair shaft in our LC-MS/MS based method (10). Others have described a wash-out effect in HCC as well (6, 66). Therefore, wash-out of hair steroids is an important consideration, especially in studies involving retrospective timelines using segmental hair analysis.

It is not yet known how cortisol enters the hair shaft. It is assumed that cortisol enters the hair shaft through passive diffusion, and that HCC therefore represent cumulative free circulating cortisol levels. This is further supported by the circumstantial evidence that oral contraception, which is known to increase total cortisol levels due to the effect of estrogens on corticosteroid binding globulin levels (2), does not seem to have a major influence on HCC (33, 67). However, active transport of steroids from the blood stream to the hair follicle cannot be excluded. Apart from incorporation from blood, sebum and sweat could also contribute to HCC. Cortisol has been demonstrated in human sweat, and it has been suggested that increased sweating may increase HCC, which may acutely influence HCC and therefore undermine the validity of HCC as a marker of long-term cortisol exposure (68). However, Stalder *et al.* recently showed that two sweat-inducing interventions (exercise and sauna bathing) do not seem to acutely influence HCC (69).

Multiple studies have measured both cortisol and cortisone in scalp hair using LC-MS/MS (10, 28). Interestingly, in a study by Stalder *et al.* (28) hair cortisone levels correlated stronger with cardiovascular risk factors than hair cortisol did. This suggests that hair cortisone levels offer valuable information about systemic glucocorticoid exposure. Furthermore, determination of both cortisol and cortisone in hair may provide information about the activity of 11β -hydroxysteroid dehydrogenase (11β -HSD) enzymes, which convert the active cortisol into cortisone and vice versa. However, 11β -HSD enzymes are expressed in skin as well (70), and may therefore impact the ratio between cortisol and cortisone in hair locally, potentially limiting the value of this ratio as a marker of systemic 11β -HSD activity.

Medication use is known to influence different types of cortisol measurements (2), but until now this does not seem to be a major limiting factor in HCC measurements. It is important however, to consider the use of topical

steroids which may contaminate hair samples, and falsely increase HCC levels through cross-reactivity in an immunoassay. In general, topical or inhalation corticosteroids may also exert some systemic effects, and thereby decrease HCC. One study described that corticosteroid use, which was not further specified, increased HCC (64). LC-MS/MS is not limited by cross-reactivity, but false increases may still occur with synthetic corticosteroids that are structurally similar to cortisol.

Future directions

Scalp hair analysis is a promising and rapidly developing additional tool to evaluate HPA axis activity. One important practical advantage is that hair sampling can easily be performed in an outpatient setting, and is not dependent on patient adherence to sampling instructions (as with 24 h urine collections or LNSC) or medication intake (as in DST). Furthermore, the ability to create retrospective timelines creates the opportunity to investigate patterns of cortisol exposure for months up to years back in time, with the collection of a single hair sample. For HCC to become a fully established method in clinical practice, findings concerning Cushing's disease need to be replicated. It should furthermore be considered that causes of pseudo-CS such as alcohol use (25, 26, 71), obesity (29) and depression (39) have also been associated with increased HCC.

In observational research, the association between increased HCC and an adverse cardiometabolic risk profile appears to be the most consistent clinical finding. All published studies which investigated this association thus far are cross-sectional, and therefore no conclusions about causal inferences can be made. Because an extreme glucocorticoid excess (i.e. CS) is strongly associated with metabolic syndrome components (central adiposity, insulin resistance, dyslipidemia and hypertension), an increased long-term cortisol exposure within the normal range is theoretically likely to have a causative role in increasing cardiovascular risk. However, the possibility of reverse causality cannot be discarded. For instance, chronic stress and increased psychopathology associated with obesity (72), or altered steroid metabolism could lead to increased cortisol levels. Therefore, there is a clear need for longitudinal studies examining the influence of long-term cortisol exposure on cardiovascular risk.

Measurement of hair cortisol creates an unique opportunity to study the effects of interventions on long-term cortisol due to the possibility of creating

retrospective timelines by dividing hair samples into segments. Randomized controlled trials could easily incorporate this method, because depending on the length of the hair, a single hair sample may be used to evaluate changes in cortisol production over multiple months. Currently, several published examples of this type of study exist. In 18 participants who underwent a mindfulness or cognitive behavioral intervention for smoking cessation, Goldberg *et al.* (73) showed a significant decrease in HCC between the hair segments corresponding to the period before the intervention, and the period after the intervention. The decrease was present in both treatment arms, and since no placebo group was present, a conclusion about the cause of the observed decrease cannot be drawn. In contrast, in a randomized clinical trial involving 261 patients with structural heart disease, we observed no difference in between patient who underwent a mindfulness based stress reduction intervention, compared to patients who were assigned to the control group, although the whole study population on average showed a decrease in HCC during the study (32). Future studies will show if, and which behavioral and medical interventions are capable of modulating long-term cortisol exposure as measured with HCC. Evidence of observational studies indicate that a decrease may be associated with health benefits, especially in cardiometabolic health.

Conclusions

Analysis of cortisol in scalp hair has provided researchers with a readily applicable tool to measure cortisol exposure over extended periods of time. Studies in the past years indicate that hair cortisol may provide a new tool to investigate the HPA axis activity in clinical practice, such as in (cyclic) CS and hydrocortisone replacement therapy monitoring. Scalp hair offers of an easy collection procedure, and importantly, the addition of long-term retrospective information of cortisol exposure. Hair cortisol levels have been shown to be altered in association with a wide array of stressors and pathological conditions. Hair analysis has shown consistent associations between increased long-term cortisol and an adverse cardiometabolic risk profile, which may pave the way towards a new method for future cardiovascular risk stratification and evaluation of treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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