SHORT COMMUNICATION

Hair cortisol and cortisol awakening response are associated with criteria of the metabolic syndrome in opposite directions

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Summary Findings on the association between hypothalamic–pituitary–adrenal (HPA) axis activity and metabolic risk are equivocal. Different methods of measuring HPA activity might indicate adverse vs. beneficial effects of HPA activity on metabolic risk thus contributing to heterogenous findings. In this study, we aimed to determine whether (1) the salivary cortisol awakening response (CAR) as a marker of awakening-induced activation of the HPA axis and (2) hair cortisol as a marker of long-term cortisol secretion are associated with criteria of the metabolic syndrome. Therefore, we recruited 41 healthy individuals (26 women, mean age: 41.2 years) and 44 patients with major depression (28 women, 41.4 years) and assessed CAR and hair cortisol values as well as all criteria of the metabolic syndrome (abdominal obesity, blood pressure, plasma glucose, triglycerides and high-density cholesterol levels) according to the International Diabetes Federation. CAR and hair cortisol values were divided into tertiles. Across groups, participants with hair cortisol or hair cortisone in the highest tertile showed significantly more criteria of the metabolic syndrome compared to participants in the medium or low tertile (F2,84 = 3.37, p = .04). These results were corroborated by significant positive
1. Introduction

The metabolic syndrome (MetS) increases the risk of cardiovascular diseases and is defined by the International Diabetes Federation as central obesity and any two of the following criteria: raised fasting plasma glucose, raised triglycerides, reduced HDL cholesterol, and raised blood pressure (Alberti et al., 2005). Altered activity of the hypothalamus–pituitary–adrenal (HPA) axis and resulting changes of cortisol release have been suggested to play a role in the development of metabolic syndrome (Walker, 2006). However, results with regard to the association between endogenous cortisol levels and the MetS are equivocal (Quax et al., 2013).

Recently, hair analysis for steroids has been introduced, which allows retrospective assessment of cumulative cortisol levels over several months (Russell et al., 2012). Interestingly, there appears to be a consistent association between higher cortisol levels and metabolic and cardiovascular risk (Manenschijn et al., 2013; Stalder et al., 2013; Veldhorst et al., 2014; Wester et al., 2014). In addition, one of these studies reports relevant associations of hair cortisol with cardiometabolic parameters (Stalder et al., 2013). Although hair cortisol has received less attention so far, it has been suggested that hair cortisol may potentially also hold merit as a relevant additional measure (Raul et al., 2004; Stalder et al., 2013).

Another approach to measure dynamic HPA axis activity is to determine the cortisol awakening response (CAR). Importantly, a blunted rather than a high CAR has been associated with unfavorable metabolic risk profiles in healthy individuals (Lasikiewicz et al., 2008; Rosmond and Björntorp, 2000), in patients with type 2 diabetes (Bruehl et al., 2009), hypertension (Wirtz et al., 2007) and depression (Lamers et al., 2013), although not all studies concur (Bengtsson et al., 2010; Kajantie et al., 2004).

These results indicate that it may depend on the technique of cortisol measurement whether a positive or negative association of HPA activity with MetS risk is found. However, no study has intra-individually investigated associations of hair cortisol and CAR with MetS at the same time. Therefore, we examined the associations of CAR as a marker of the characteristic awakening-induced reactivity of the HPA axis and hair cortisol and hair cortisone as markers of long-term cortisol levels with MetS criteria. Based on prior studies, we expected that CAR would be negatively associated with metabolic risk while hair cortisol and hair cortisone would be positively associated with metabolic risk.

2. Material and methods

2.1. Participants

We recruited 44 depressed patients according to DSM-IV criteria (for demographic and clinical variables see Table 1a) and 41 age- and sex-matched healthy subjects.

Criteria for exclusion in the depressed group were dementia, schizophrenia spectrum disorder, bipolar disorder, substance dependence, serious medical conditions associated with adrenal dysfunction, steroid use (including oral intake, crèmes, nasal sprays, inhalation medications, intra-articular injections), or well-known impact correlations between mean hair cortisol values with waist circumference ($r = .29, p = .03$), triglycerides ($r = .34, p = .01$) and systolic blood pressure ($r = .29, p = .04$) and between mean hair cortisol and triglycerides ($r = .46, p < .01$). In contrast, mean CAR values correlated negatively with diastolic ($r = -.29, p = .03$) and systolic blood pressure ($r = -.32, p = .02$). Our results indicate that higher hair cortisol and hair cortisone levels but lower CAR values are associated with an unfavorable metabolic and cardiovascular risk profile.

Table 1a Descriptive information of the study sample (mean and standard error).

<table>
<thead>
<tr>
<th></th>
<th>Healthy participants</th>
<th>MDD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (n = 26)</td>
<td>Men (n = 15)</td>
</tr>
<tr>
<td>Hair cortisol (pg/mg)</td>
<td>3.3 (0.6)</td>
<td>6.1 (1.4)</td>
</tr>
<tr>
<td>Hair cortisone (pg/mg)</td>
<td>14.6 (2.1)</td>
<td>28.8 (4.9)</td>
</tr>
<tr>
<td>Cortisol awakening response (AUCg)</td>
<td>552.7 (27.8)</td>
<td>420.4 (26.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.7 (2.3)</td>
<td>38.7 (3.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 (0.6)</td>
<td>25.5 (0.9)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.6 (1.7)</td>
<td>94.5 (3.0)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121.4 (3.3)</td>
<td>130.7 (4.5)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.7 (2.2)</td>
<td>78.1 (2.7)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>71.9 (3.2)</td>
<td>82.4 (3.5)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>62.4 (2.3)</td>
<td>49.8 (5.7)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>106.7 (12.1)</td>
<td>196.3 (59.9)</td>
</tr>
<tr>
<td>BDI</td>
<td>2.4 (0.5)</td>
<td>1.2 (0.5)</td>
</tr>
<tr>
<td>MADRS</td>
<td>—</td>
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on hypothalamic–pituitary–adrenal (HPA) activity, pregnancy, and nursing. Twenty-four patients were free of psychotropic medication, while 20 patients were treated with selective serotonin reuptake inhibitors (n = 8), selective norepinephrine reuptake inhibitors (n = 2), selective serotonin norepinephrine reuptake inhibitors (n = 3), mirtazapine (n = 3), agomelatine (n = 2), amitriptyline (n = 2), St. John’s wort (n = 2), tranylcypromine (n = 1) and opipramol (n = 1). A total of six used medication of the group of β-blockers (n = 2), ACE-/AT-blockers (n = 5) or diuretics (n = 1). The depressed patients (27 in- and 17 out-patients) were recruited from specialized depression clinics at the Department of Psychiatry and Psychotherapy and the Department of Psychosomatic Medicine, University Medical Center Hamburg (Germany) and had a mean BDI (Beck’s Depression Inventor) score of 32.2 (SD: 1.4) and a mean MADRS (Montgomery-Asberg Depression Rating Scale) score of 30.1 (SD: .9). For about one third of the patients (36.4%) the current episode was the first one.

Healthy participants were free of former and present DSM-IV axis I disorders as assessed by the Mini International Neuropsychiatric Interview, had no physical illness apart from one case of medically adjusted hypertension, and were free of any medication.

Across groups, exclusion criteria were examined by the MINI Interview, a physical exam, a clinical interview and a blood count.

The study was approved by the local ethics committee, and written informed consent was obtained.

2.2. Hair cortisol and hair cortisone

Hair strands of a total diameter of approximately 3 mm were taken from the scalp and cut into 3-cm segments (reflecting the cumulative hormone secretion over a 3-month period) as described by Steudte et al. (2013). Wash and steroid extraction procedures followed the protocol described in Stalder et al. (2012). Intra-assay and inter-assay coefficients of variance have been shown to be between 3.7% and 8.8% (Gao et al., 2013). Due to technical reasons, hair samples of three participants (1 patient, 2 healthy participants) could not be analyzed.

2.3. Cortisol awakening response

Salivary cortisol was collected on two consecutive days, preferable on working days, at awakening, +30 min and +60 min after awakening using Salivette devices (Sarstedt, Rommelsdorf, Germany). Cortisol was determined by radioimmunoassay (DRG Diagnostics GmbH, Germany). Inter- and intra-assay coefficients of variation were below 8%. Detection limits were 0.5 ng/ml for cortisol.

2.4. Metabolic syndrome

We used the MetS definition of the International Diabetes Federation (Alberti et al., 2005): central obesity measured by waist circumference (men > 94 cm, women > 80 cm using ethnicity specific values for Europids), raised triglycerides (> 150 mg/dL or specific treatment), reduced high-density lipoproteins-cholesterol (HDL; < 40 mg/dL in men, < 50 mg/dL in women, or specific treatment), raised blood pressure (BP; systolic: ≥ 130 mmHg, diastolic: ≥ 85 mmHg, or treatment of previously diagnosed hypertension) and raised fasting plasma glucose (≥ 100 mg/dL, or previously diagnosed type 2 diabetes). Across groups, blood samples were taken under fasting conditions between 8 and 9.30 AM. Additionally, weight, height, and waist circumference were assessed and blood pressure was recorded after 5 min of rest. All participants were requested to avoid physical activity such as biking before examination. All blood samples were analyzed in the laboratory of the University Medical Center Hamburg (Germany).

2.5. Statistical analysis

The cortisol awakening response (CAR) was calculated as the area under the curve with respect to ground (AUCg) from salivary cortisol data of two consecutive days.

Levels of hair cortisol, hair cortisone and CAR were divided into tertiles. Differences in the number of fulfilled MetS criteria according to tertiles were calculated using one-way ANCOVAs. Differences in the present of MetS (central obesity plus at least two other fulfilled MetS criteria) according to tertiles were calculated by X²-tests.

Associations of hair cortisol, hair cortisone and CAR with MetS criteria (waist circumference, BP, glucose, HDL, triglycerides) were examined using partial correlation analyses controlling for potentially confounding variables. We examined potentially confounding variables using two-tailed t-tests for dichotomous variables (sex, diagnosis, smoking) and correlation analyses for continuous variables (age, education, depressive symptoms) with hair cortisol, hair cortisone or CAR.

A p-value smaller than .05 was considered to indicate statistical significance, for correlation analyses also significance at trend (p < .10) is reported.

3. Results

There was no significant difference in hair cortisol, hair cortisone or CAR between the depressed and the healthy group (hair cortisol: t₁ = 1.29, p = .20; hair cortisone: t₁ = .28, p = .78; CAR: t₁ = .71, p = .48).

Examining potentially confounding variables, we found no significant differences according to diagnosis of major depression, age, smoking or education. There were significant sex differences of hair cortisol levels (t₁ = 2.20, p = .03) and CAR (t₁ = 2.39, p = .02), but not of hair cortisone levels. Thus, ’’sex’’ was included as control variable in all ANCOVAs and partial correlations.

3.1. Hair cortisol and hair cortisone

We found significant differences between the tertiles of hair cortisol on fulfilled MetS criteria (F₁,64 = 3.37, p = .04). Participants in the highest tertile showed a significant higher number of fulfilled MetS criteria compared to the medium and low tertile (mean: 2.4, SD: .3 vs. mean: 1.3, SD: .3 in the medium tertile and mean: 1.4, SD: .3 in the
Bonferroni corrected post hoc analyses demonstrated significant differences of fulfilled MetS criteria in the highest tertile vs. medium tertile \((p < .05)\) and on trend level significance vs. lowest tertile \((p = .07, \text{ see Fig. } 1)\). Significant differences in the present of MetS according to tertiles were revealed by \(X^2\)-test showing that the highest number of MetS was present in the highest tertile \((X^2 = 7.35, p = .03)\); low tertile: 3 subjects with MetS, 20 without MetS vs. high tertile: 10 with MetS, 11 without MetS).

Sex-adjusted bivariate correlations showed that hair cortisol levels were positively associated with waist circumference \((r = .29, p = .03)\), triglycerides \((r = .34, p = .01)\), and systolic blood pressure \((r = .29, p = .04, \text{ see Table } 1b)\). Additionally, hair cortisol levels were significantly correlated with hair cortisone levels \((r = .83, p < .01, \text{ see Table } 1b)\).

Results of hair cortisone were confirmed by results of hair cortisol. There was a significant difference for the number of fulfilled MetS criteria between tertiles on trend-level \((F_{2,64} = 2.69, p = .08)\). Participants in the highest tertile showed a higher number of fulfilled MetS criteria compared to the medium and low tertile (mean: 2.2, SD: .3 vs. mean: 1.7, SD: .3 in the medium and mean: 1.1, SD: .2 in the low tertile, see Fig. 1). Bonferroni corrected post hoc analyses revealed a significant difference between the numbers of fulfilled MetS criteria in the highest tertile vs. the lowest tertile \((p < .04, \text{ see Fig. } 1)\). When we combined hair cortisol and hair cortisone to total hair glucocorticoid levels, results for fulfilled MetS criteria according to tertiles did not change. Significant differences in the present of MetS according to tertiles of hair cortisone levels were revealed by \(X^2\)-test showing that the highest number of MetS was present in the highest tertile \((X^2 = 7.16, p = .03)\); low tertile: 2 subjects with MetS, 21 without MetS vs. high tertile: 10 with MetS, 13 without MetS).

Sex-adjusted bivariate correlations showed that hair cortisone levels were positively associated with triglycerides \((r = .46, p < .01)\) and on trend level with waist circumference \((r = .25, p = .07, \text{ see Table } 1b)\).

3.2. Cortisol awakening response (CAR)

Number of fulfilled MetS criteria did not differ between tertiles of CAR \((F_{2,63} = 0.90, p = .42)\), although the highest number of fulfilled MetS criteria was found in the lowest tertile (mean: 1.8, SD: .3 vs. mean: 1.4, SD: .3 in the medium tertile vs. mean: 1.3, SD: .2 in the high tertile, see Fig. 1). There were no significant differences in the present of MetS according to tertiles \((X^2 = 2.66, p = .29)\), although the highest number of MetS was present in the lowest tertile (6 subjects with MetS, 15 without MetS vs. high tertile: 2 with MetS, 20 without MetS).

Sex-adjusted bivariate correlation analyses showed that CAR was negatively associated with measures of blood pressure (systolic BP: \(r = −.29, p = .03\), diastolic BP: \(r = −.32, p = .02\)). There was no significant association of CAR with hair cortisol \((r < .01, p = .96)\). There was no significant association of CAR with hair cortisone \((r = .18, p = .20, \text{ see Table } 1b)\).

4. Discussion

We found that, across groups, participants in the highest tertile of hair cortisol and in the highest tertile of hair cortisone fulfilled more criteria of the metabolic syndrome (MetS) compared to participants in the medium or low tertile. Additionally, MetS was more often present in participants in the highest tertile of hair cortisol and in the highest tertile of hair cortisone. Furthermore, hair cortisol correlated positively with waist circumference, triglycerides, and systolic blood pressure and hair cortisone with triglycerides. In contrast, CAR correlated negatively with systolic and diastolic blood pressure.

These apparently contrary results of low and high cortisol measures to be associated with an enhanced MetS risk may be explained when CAR and hair cortisol are regarded as rather independent markers of HPA axis activity. Indeed, the
The magnitude of CAR was not associated with hair cortisol in our study.

Hair cortisol reflects long-term cortisol levels and is considered a more reliable method than a single time point saliva or blood measurement, because influences of the circadian rhythm, release pulses and acute stress can be minimized. Our results of hair cortisol are in accordance with the literature showing consistent evidence for an association of hair cortisol and MetS. Stalder et al. (2013) reported a higher prevalence of MetS in participants with high hair cortisol levels and positive correlations of hair cortisol and hair cortisone with cardiometabolic risk factors. Another study demonstrated an increased risk for cardiovascular disease and type 2 diabetes in elderly participants with high hair cortisol levels (Manenschijn et al., 2013). This result is supported by a study in obese compared to normal-weight children (Veldhorst et al., 2014). As reported in further studies (Raul et al., 2004; Stalder et al., 2013), levels of hair cortisone showed higher absolute values than hair cortisol. Local conversion may play a major role in determining the relative levels of hair cortisol and hair cortisone as 11-beta-hydroxysteroid dehydrogenase enzymes that interconvert cortisol and cortisone are expressed in specific cell types in human skin (Smith et al., 1996; Tiganescu et al., 2011). Our results show that levels of hair cortisone and hair cortisol were closely associated and showed a similar correlation pattern with MetS criteria. Regarding the analyses of tertiles and fulfilled MetS criteria, hair cortisone levels seem to reflect a more pronounced dose-dependent effect compared with hair cortisol levels. It has been suggested that hair cortisol and hair cortisone generally convey similar information but that, for some individuals, hair cortisone may provide a closer and more robust reflection of systemic cortisol levels as it has been described for salivary data before (Perogamvros et al., 2010; Stalder et al., 2013). However, future methodological research is clearly needed for a proper interpretation of hair cortisone.

While hair cortisol and hair cortisone reflect long-term cortisol secretion, CAR is a dynamic measure of HPA axis (re-) activity. Thus, a blunted CAR may reflect impaired HPA axis activity. A blunted CAR, as part of an impaired HPA axis profile, has even been suggested as an additional marker of metabolic vulnerability (Lasikiewicz et al., 2008). Accordingly, most previous studies found a blunted CAR to be associated with higher metabolic risk (Bruehl et al., 2009; Lamers et al., 2013; Lasikiewicz et al., 2008) although not all results are consistent (Bengtsson et al., 2010; Kajantie et al., 2004). In line with the majority of the previous studies, we found that higher CAR values were associated with higher carotid and systolic blood pressure values. This is also in line with a study (Wirtz et al., 2007), in which hypertensive participants showed a lower CAR compared to normotensives. Of course, CAR is influenced by a variety of factors such as cortisol pulsatility, daily variation, acute stress, and time of waking up. We controlled for these factors to some extend by using samples of two consecutive working days.

Due to the cross-sectional design of our study, no firm conclusion can be drawn regarding the direction of association between cortisol values and MetS criteria. However, there is a plethora of evidence prospectively demonstrating that cortisol affects metabolic and cardiovascular risk. Accordingly, hypercortisolism as in Cushing’s syndrome is characterized by central obesity, hypertension, glucose intolerance and dyslipidaemia (Walker, 2006). However, MetS criteria may in turn affect HPA axis activity. We controlled for potentially confounding variables such as age or sex. However, we cannot completely exclude residual confounding. The relatively small sample size may account for the fact that some of the results were only significant at trend level.

In summary, we found that high hair cortisol and high hair cortisone but low values of CAR were associated with an enhanced MetS risk. Whether lowering long-term cortisol elicits beneficial effects on metabolic risk needs to be determined longitudinally.
Role of the funding source

None.

Conflict of interest statement

There are no conflicts of interest, financial or otherwise, to declare.

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References


