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Chapter 7

Differences in the response of the hypothalamic-pituitary-adrenal axis in presymptomatic mutation carriers of Huntington’s disease in comparison to symptomatic mutation carriers and controls

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Submitted

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Abstract
Neurodegeneration in Huntington’s disease occurs in various brain regions including the hypothalamus. In this cross-sectional study, hypothalamic-pituitary-adrenal axis functioning was studied in 26 presymptomatic and 58 symptomatic Huntington’s disease mutation carriers, and 28 controls. Hypothalamic-pituitary-adrenal axis functioning was measured through salivary cortisol in the day curve, the cortisol awakening response (CAR), the area under the curve (AUC), the morning rise, and the dexamethasone suppression test (DST). The CAR was statistically different (p = 0.046) between the three groups, being explained by higher cortisol concentrations at 45 and 60 minutes post-awakening for presymptomatic mutation carriers compared to both symptomatic mutation carriers and controls. The morning rise was also higher for presymptomatic mutation carriers (p = 0.005). No differences were found for the AUC, evening and post-DST cortisol concentrations. Our study indicates a delicate disturbance in morning cortisol secretion in Huntington’s disease mutation carriers that precedes the onset of motor symptoms.

Introduction
Huntington’s disease is a progressive autosomal dominant neurodegenerative disorder characterized by motor symptoms, cognitive decline, behavioral problems and psychiatric disorders. Huntington’s disease is caused by a trinucleotide expansion on chromosome 4 (4p16.3), coding for the mutant protein huntingtin.7 Neurodegeneration primarily occurs in the striatum and cerebral cortex. Atrophy has also been found in hypothalamic areas,14 with neuronal loss up to 90% in the nucleus tuberalis lateralis.15,16 Direct involvement of huntingtin and pathological mechanisms, such as decreased hypocretin neurotransmission,7 loss of hypothalamic D1 receptors and microglia activation,7 may play a role in hypothalamic dysfunctioning in Huntington’s disease. Consequently, malfunctioning of the hypothalamic-pituitary-adrenal axis might occur.14

The hypothalamic-pituitary-adrenal axis regulates the stress response.10 Corticotropin-releasing hormone, being released in a circadian, pulsatile rhythm in the hypothalamus with an increase in amplitude in the early morning hours, stimulates the anterior pituitary to produce adrenocorticotropic hormone that triggers the secretion of glucocorticoids from the adrenal cortex.

Previous studies have reported a hyperactivation of the hypothalamic-pituitary-adrenal axis in mutation carriers with increased corticotropin-releasing hormone in cerebrospinal fluid (CSF),11 and increased cortisol concentrations in plasma,12,13 and urine.14 However, none of these studies, except for one,15 took into account the circadian rhythm of the hypothalamic-pituitary-adrenal axis. Sample sizes varied from 10 to 82 Huntington’s disease mutation carriers, while potential confounders of the hypothalamic-pituitary-adrenal axis were inconsistently taken into account. Our study therefore aimed to investigate the functioning of the hypothalamic-pituitary-adrenal axis as assessed with a cortisol day curve and dexamethasone suppression test (DST) in presymptomatic and symptomatic Huntington’s disease mutation carriers, compared to controls.

Experimental procedures
Subjects
All 210 participating subjects (154 Huntington’s disease mutation carriers and 56 controls) of an ongoing follow-up study on behavioral problems and psychiatric disorders in Huntington’s disease,13 were invited to participate. These persons had been recruited at the start of the study from the outpatient clinics of Neurology and Clinical Genetics of the Leiden University Medical Center, a nursing home with a specialized ward for Huntington’s disease patients and the Dutch Huntington’s disease patients association. Verified non-carriers with a CAG repeat < 36 were included as a control group because they had been exposed to the same stressful family circumstances as mutation carriers. Severely dysarthric and mutistic patients were excluded, as well as patients with juvenile onset Huntington’s disease, concurrent diseases of the central nervous system or an insufficient command of the Dutch language. All subjects were Caucasian.
Twenty-five subjects refused to participate in this follow-up study, four subjects were untraceable, two were deceased, and one subject had become too severely affected to communicate. The remaining 178 subjects participated in this part of the study. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and all subjects gave their informed consent.

**Demographic and clinical characteristics**

Demographic and clinical characteristics, including potential confounders for hypothalamic-pituitary-adrenal axis functioning, like sex, age, smoking status, high alcohol consumption (> 14 consumptions a week), body mass index (BMI), presence of depressive disorder, and use of corticosteroid and psychotropic medication were assessed during a standardized interview. In addition, global cognitive functioning and general functioning were measured.

The presence of a depressive disorder (major depressive or dysthmic disorder) in the past two weeks was assessed with the Composite International Diagnostic Interview (CIDI), computerized version 2.1.14 The CIDI is a fully structured psychiatric interview for disease classification of psychiatric disorders according to the Diagnostic and Statistical Manual of mental disorders (DSM).15 Global cognitive functioning was measured using the Mini-Mental State Examination (MMSE).15 Because of lack of reliability in subjects with severe cognitive dysfunction, the CIDI was not administered to subjects with a MMSE score < 18 points. Global general functioning was assessed using the Total Functioning Capacity (TFC) of the Unified Huntington’s Disease Rating Scale (UHDRS).15 The TFC consists of 5 questions assessing employment, the capacity to handle financial affairs, to manage domestic chores, to perform activities of daily living, and the care level provided. The TFC ranges from 0 - 13 points, with lower scores indicating poorer functional abilities.20

**Assessment of motor functioning and disease stage**

Subjects were examined for assessment of motor symptoms by a neurologist with experience of Huntington’s disease using the motor section of the UHDRS. The neurologist was blinded to the genetic status of the subjects and the results of all other assessments. Based on the clinical examination, the neurologist assigned a score indicating to what degree he was confident that the presence of an extrapyramidal movement disorder in a subject might be due to Huntington’s disease. Mutation carriers with confidence level score 0 (normal) or 1 (nonspecific motor abnormalities; < 50% confidence) were considered presymptomatic (n = 26). The remaining mutation carriers (n = 58) with score 2 (motor abnormalities that may be signs of Huntington’s disease; 50 - 89% confidence), 3 (likely signs of Huntington’s disease; 90 - 98% confidence), or 4 (unequivocal signs of Huntington’s disease; ≥ 99% confidence) were considered symptomatic.

**Measurement of hypothalamic-pituitary-adrenal axis functioning**

Functioning of the hypothalamic-pituitary-adrenal axis was assessed by the use of cortisol concentrations in saliva, reflecting the free fraction of plasma cortisol.22 Advantages of salivary cortisol above plasma cortisol measurement are the easy collection of saliva by the subjects at their homes, the possibility of repeated sampling to yield a day curve, the stability of cortisol at room temperature during the time required for this study, the absence of stress induction by a venupuncture, and the lower costs.22 After oral and written instruction, subjects were asked to collect saliva by themselves on two consecutive days. For this, they had to place cotton wads from a saliva collection tube (Salivette; Sarstedt, Newton, NC) in their mouth and chew on them until they were saturated. The wads were restored in the tube labeled with date and time. Subjects were asked to refrain from eating, drinking, and brushing their teeth before the morning sampling to avoid contamination of the saliva with food or blood. They were free to wake up according to their normal schedule, but were asked to record their time of awakening because the cortisol response may be influenced by the time of awakening.23

The circadian rhythm of the hypothalamic-pituitary-adrenal axis was taken into account by assessing a cortisol day curve. On day 1 six samples were taken: at the time of awakening, 30, 45, and 60 minutes post-awakening, at 22:00 h, and at 23:00 h. The cortisol awakening response (CAR) is a distinctive measurement of the cortisol circadian cycle. In healthy adults salivary cortisol concentrations increase by 50% to 160% in the first 30 minutes post-awakening.23 The CAR is defined as the mean of the two cortisol concentrations at 45 minutes and at 60 minutes post-awakening, minus the cortisol concentration at the time of awakening on day 1.23 The area under the curve (AUC) with respect to ground was calculated according to the trapezoid formula using the first four time points.23

The DST is a measure of hypothalamic-pituitary-adrenal axis regulation and normally shows a decrease of morning cortisol concentrations due to inhibition of adrenocorticotropin hormone secretion after dexamethasone administration the night before.24 A low dose of dexamethasone (0.5 mg) had to be taken orally after the last sample on day 1, and the final sample was taken at the time of awakening on day 2. After collecting all seven samples, the subjects were asked to return the tubes through regular postal service. After centrifugation of the cotton wad, salivary cortisol concentrations were measured with a competitive electrochemiluminescence immunoassay (ECLIA), using a Modular Analytics E170 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) by the Central Laboratory for Clinical Chemistry of the Leiden University Medical Center. The functional detection limit was 2.0 nmol/l and the intra- and inter-assay variability coefficients in the measuring range were less than 10%. We assumed concentrations ≥ 100 nmol/l to be physiologically unlikely.

**Statistical analyses**

Categorical variables are presented as numbers and percentages, and continuous variables as means ± standard deviations (SD) or medians with percentiles P25 - P75, when appropriate. Differences between the three groups were assessed by one-way analysis of variance (ANOVA). Post-hoc intergroup comparisons were performed for those variables with significant test results. All subjects with four or more missing salivary cortisol concentrations on day 1 were excluded (n = 5). All other missing cortisol data (n = 21 of 672; 3.1%) were extrapolated by using the subject’s preceding and following salivary cortisol values, and modeling the average curve from all subjects over these values for that point in time. For positively skewed variables, natural log-transformed values were used in statistical analyses, and back-transformed geometric mean values are presented in tables. The cortisol awakening response (CAR) was analyzed by repeated measurements general linear models (GLM), with time as within-subject factor and
group as between-subject factor. Next, three markers of salivary cortisol were calculated. The total area under the curve of the morning rise (AUC) was calculated using trapezoid formula. The morning rise during the CAR was calculated as the maximum of the two cortisol concentrations at 30 minutes and at 45 minutes post-wakening, minus the cortisol concentration at time of awakening on day 1. The cortisol suppression ratio was calculated as the salivary cortisol at the time of awakening on the first day / post-DST salivary cortisol. Differences in cortisol levels and markers between the three groups were assessed by ANOVA. The covariates sex, age, the use of psychotropic medication, and the time of awakening were added to multivariable models using ANOVA, to adjust for potential confounding effects. Significance levels were set at $p < 0.05$. Statistical analysis was performed using the Statistical Package for the Social Sciences 16.0 for Windows.

Results
Demographic and clinical characteristics
Forty subjects (22%) declined saliva collection, 24 subjects (13%) collected insufficient saliva, and two subjects (1%) were excluded because of physiologically unlikely high salivary cortisol concentrations. One subject was excluded because of the use of oral corticosteroid medication at the time of the study. None of the female subjects reported pregnancy, that potentially may affect cortisol concentrations. This resulted in available saliva of 58 symptomatic and 26 presymptomatic mutation carriers, and 28 controls. Demographic and clinical characteristics of these subjects are presented in Table 1; as is shown, data of the potential confounders smoking status, alcohol consumption, BMI, and use of psychotropic medication were incomplete. Further, the presence of a depressive disorder could not be assessed in five symptomatic mutation carriers because of a MMSE score < 18 points. Hypothalamic-pituitary-adrenal axis functioning
Since we assumed cortisol concentrations $\geq 100$ nmol/l to be physiologically unlikely, measurements at seven time points (1.0%) of a total of 672 measurements in 112 subjects were excluded.

On day 1, using repeated measurements GLM there was a time effect for the CAR ($p < 0.001$, indicating an increase at 30 minutes post-wakening), a time * group effect ($p = 0.04$, indicating that the dynamics of the curve were dissimilar between the groups), and a group effect ($p = 0.046$). Post-hoc tests showed that the mean cortisol concentration of the presymptomatic mutation carriers was higher compared to the symptomatic mutation carriers ($p = 0.035$), largely due to higher mean cortisol concentrations at 45 minutes and 60 minutes post-wakening (Table 2 and Figure 1). For the mean evening cortisol concentrations there was no time effect ($p = 0.29$), no time * group effect ($p = 0.95$), and no group effect ($p = 0.21$). The AUC showed a trend towards significance ($p = 0.09$).

On day 2, using ANOVA after the DST, there were no statistically significant differences between the groups for morning salivary cortisol concentrations ($p = 0.39$) nor for the cortisol suppression ratio ($p = 0.92$) (Table 2).

Effect of potential confounders
The three groups did not differ for smoking status, alcohol consumption, BMI, and the presence

| Table 1. Demographic and clinical characteristics of all subjects (n = 112) |
|----------------|----------------|----------------|----------------|
| Demographic characteristics |  |  |  |
| Male (n, %) | 106 | 100 | 112 |
| Female (n, %) | 54 | 40 | 58 |
| Age (mean ± SD) | 55.9 ± 13.9 | 68.6 ± 11.7 | 59.8 ± 12.9 |
| Clinical characteristics |  |  |  |
| Smoker (n, %) | 37 | 30 | 42 |
| High alcohol consumption (n, %) | 21 | 20 | 22 |
| BMI (mean ± SD) | 26.3 ± 3.6 | 27.1 ± 4.5 | 26.2 ± 3.6 |
| Depressive medication (n, %) | 2 | 0 | 2 |
| MMSE (median) | 29 | 28 | 28 |
| TFC (median) | 13 | 12 | 13 |

- *Significant at $p < 0.05$.
### Table 2. Mean salivary cortisol concentrations and derived cortisol curve parameters of all study subjects (n = 112)

<table>
<thead>
<tr>
<th>Cortisol day curve (nmol/L)</th>
<th>Presymptomatic mutation carriers (n = 26)</th>
<th>Symptomatic mutation carriers (n = 58)</th>
<th>Controls (n = 28)</th>
<th>p value (crude)</th>
<th>p value (adjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of awakening</td>
<td>18.6 (10.0 - 31.6)</td>
<td>18.8 (11.2 - 33.1)</td>
<td>16.5 (9.2 - 29.3)</td>
<td>0.57</td>
<td>0.23</td>
</tr>
<tr>
<td>+ 30 minutes</td>
<td>23.6 (13.0 - 37.5)</td>
<td>18.7 (9.2 - 36.9)</td>
<td>19.4 (7.9 - 35.0)</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>+ 45 minutes</td>
<td>24.6 (13.4 - 56.3) *</td>
<td>17.6 (8.1 - 37.4)</td>
<td>18.0 (8.5 - 30.8)</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>+ 60 minutes</td>
<td>19.8 (8.3 - 48.7) *</td>
<td>14.0 (6.2 - 28.0)</td>
<td>14.1 (4.9 - 24.3)</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>22:00 hours</td>
<td>5.4 (2.6 - 13.7)</td>
<td>4.7 (2.1 - 12.4)</td>
<td>3.9 (1.2 - 11.2)</td>
<td>0.24</td>
<td>0.34</td>
</tr>
<tr>
<td>23:00 hours</td>
<td>5.7 (1.9 - 19.0)</td>
<td>4.9 (2.2 - 11.7)</td>
<td>4.2 (1.3 - 14.3)</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>Morning rise (nmol/L)</td>
<td>9.5 (4.2 - 14.7) *</td>
<td>2.0 (0.5 - 4.6)</td>
<td>6.8 (2.1 - 11.4)</td>
<td>0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>AUC (minutes * nmol/l)</td>
<td>2.75 (1.63 - 4.69)</td>
<td>2.20 (1.23 - 3.92)</td>
<td>2.22 (1.35 - 3.62)</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>Post-OST (nmol/l)</td>
<td>6.6 (1.3 - 2.5)</td>
<td>7.6 (1.1 - 2.7)</td>
<td>6.3 (0.9 - 2.6)</td>
<td>0.39</td>
<td>0.34</td>
</tr>
<tr>
<td>Cortisol suppression ratio</td>
<td>2.9 (2.3 - 3.5)</td>
<td>2.8 (2.3 - 3.4)</td>
<td>3.0 (2.4 - 3.6)</td>
<td>0.92</td>
<td>0.96</td>
</tr>
</tbody>
</table>

AUC = Area Under the Curve for the CAR with respect to ground (minutes * nmol/l); Post-OST = cortisol concentration after the Desamethasone Suppression Test.

Geometric mean and 95% confidence intervals of the mean are given, except for the CAR and the cortisol suppression ratio for which the mean and 95% confidence intervals of the mean are given.

- *: Geometric mean and 95% confidence intervals of the mean.
- #: To avoid an increased risk of cortisol rebound, subjects are asked to refrain from taking any medications for at least 30 min before awakening.
- !: Cortisol concentration after OST was not assessed in one presymptomatic and seven symptomatic mutation carriers because they refused to take desamethasone.
- "":fairly adjusted for sex, age, psychiatric medication, and time of awakening on day 1 for the cortisol day curve, CAR, and AUC and on day 2 for the post-OST cortisol and the cortisol suppression ratio by analysis of variance.
- ***: values in the same row with different superscript letters are significantly different; p < 0.05 (post-hoc test).

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**Figure 1.** Salivary cortisol concentrations of the day curve and post-OST.

Salivary cortisol concentrations (nmol/L) were significantly higher, compared to non-carriers, as well as compared to symptomatic mutation carriers.

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**Figure 2.** Differences in the response of the hypothalamic-pituitary-adrenal axis.

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Salivary cortisol concentrations were significantly lower in the presymptomatic carriers compared to the symptomatic carriers. Differences in the response of the hypothalamic-pituitary-adrenal axis were found for age and use of psychotropic medication. Symptomatic mutation carriers woke up later after control of the morning rise during the CAR and woke up later after adjustment for age, sex, and psychotropic medication. Symptomatic mutation carriers woke up later after controls of the morning rise during the CAR. The AUC between the three groups was not significantly different (1.012 ± 1.109). The AUC between the three groups was still significantly different after exclusion of subjects with time of awakening after 5:00 h (p = 0.005).
Discussion
The differences in the CAR of the three study groups were explained by higher salivary cortisol concentrations at 45 and 60 minutes post-awakening and a higher morning rise for presymptomatic mutation carriers compared to both symptomatic mutation carriers and controls. These differences persisted after adjustment for potential confounders.

Increased basal plasma cortisol concentrations have previously been reported in two small studies including only 10,17 and 11 symptomatic Huntington’s disease patients,21 whereas no difference was found in a single morning sample between 8:00 h and 10:00 h in a study comparing 41 symptomatic and 18 presymptomatic female Huntington’s disease mutation carriers as well as healthy controls.22 Similar to our findings, no significant difference was found for post-DST cortisol concentrations between 10 Huntington’s disease patients and 10 controls.23 In a large study among 82 moderate and advanced Huntington’s disease mutation carriers, higher urinary cortisol concentrations have been described, compared to 68 healthy controls.14 However, in the latter study, measurement of cortisol concentrations was performed in urine samples that were collected during a short time period (between 14:00 and 17:00 h), and disease stage was defined according to the TFC instead of the motor section of the UHDRS. Thus, except for one small study,14 the circadian rhythm was not taken into account. Moreover, potential confounders of hypothalamic-pituitary-adrenal axis functioning such as smoking status, alcohol consumption, BMI, use of psychotropic medication and presence of depression, was inconsistently adjusted for in the four studies that examined hypothalamic-pituitary-adrenal axis functioning in Huntington’s disease.

Different hypotheses exist concerning hyperactivation of the hypothalamic-pituitary-adrenal axis in Huntington’s disease. First, psychosocial life stress from growing up in families with members suffering from Huntington’s disease might induce chronic hypothalamic-pituitary-adrenal axis hyperactivation. Second, following disclosure of being mutation carrier, presymptomatic mutation carriers may experience stress due to continuous self-observation for the onset of symptoms, causing hyperactivity of the hypothalamic-pituitary-adrenal axis. Third, hyperactivation of the hypothalamic-pituitary-adrenal axis could be the result of hypothalamic degeneration disrupting its delicate feedback mechanisms.24 Fourth, degeneration of the hippocampus and the frontal cortex in Huntington’s disease may indirectly cause a diminished feedback inhibition of the hypothalamic-pituitary-adrenal axis, leading to hyperactivation.25

There are indications that increased cortisol concentrations may cause further degeneration of the hippocampus.26,27 Fifth, it has been suggested that the loss of GABA neurons in Huntington’s disease induces an endogenous corticotropin-releasing hormone overdrive, resulting in higher cortisol levels.28 Also, increased cortisol concentrations may in turn contribute to an increased susceptibility for emotional disturbances, which may further induce hypothalamic-pituitary-adrenal axis activity.29

These hypotheses would lead one to expect a further hyperactivation of the hypothalamic-pituitary-adrenal axis during disease progression, but this is not supported by the data from our cross-sectional study. In contrary to an earlier report,14 we found diminished activation of the hypothalamic-pituitary-adrenal axis in subjects with prevalent motor symptoms reflecting more advanced disease stage. In our opinion, this might be the result of either decreased responsiveness or exhaustion of the hypothalamic-pituitary-adrenal axis, which is supported by a postmortem study that reported a decreased concentration of corticotropin-releasing hormone immunoreactivity in the striatum of 11 Huntington’s disease patients.30

Alternatively, the impact of psychosocial stress may be reduced once the disease is clinically manifest, as a result of the acceptance of the disease, whereas in advanced disease stage, subjects may have a diminished awareness of current stress factors.

Several limitations of the present study need to be addressed. First, the data are cross-sectional, and therefore do not allow for causal inferences. Second, the saliva collection was unsupervised; to improve compliance with respect to the time instructions, controlled collection using devices with electronic time registration is advised but expensive. Also, some subjects in an advanced stage of the disease had difficulties in collecting sufficient saliva, possibly as a result of disturbed osmoregulation or impaired saliva production in Huntington’s disease.31 Third, missing cortisol concentrations were extrapolated, but potential effect of bias is likely to be small as only 3% of time points were missing. Fourth, disease stage was defined according to the confidence level of the motor section of the UHDRS that depends on the experience and knowledge of the clinician, and solely assesses the presence of motor symptoms. Finally, we found differences between the groups for the CAR, but it is unclear whether other exogenous factors such as season, day of the week and sleep regulation have confounded this association.

Despite these shortcomings our study indicates a delicate disturbance in morning cortisol secretion in Huntington’s disease mutation carriers that precedes the onset of motor symptoms, and possibly plays a role in the progression of the disease. Hyperactivity of the hypothalamic-pituitary-adrenal axis may play a role in the development of the first subtle symptoms of Huntington’s disease, including psychiatric phenomena. The use of more refined rating scales might increase our insight into a potential relationship between hypothalamic-pituitary-adrenal axis activation and the early manifestation of Huntington’s disease.
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