Chapter 5

Hypothalamic-pituitary-adrenal axis functioning in Huntington's disease and its association with depressive symptoms and suicidality

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J Neuroendocrinol 2015;27(3):234-244

Abstract

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been reported in Huntington's disease (HD). In non-HD populations, alterations in HPA axis activity have been associated with depression and suicidality. The present study aims to compare HPA axis activity between HD mutation carriers and controls, and examine its association with depressive symptoms and suicidality. To this end, salivary cortisol concentrations at seven time points, as well as depressive symptoms and suicidality, were assessed in 49 pre-motor, 102 motor symptomatic mutation carriers and 55 controls, at baseline and follow-up combined. Differences in parameters of HPA axis activity between these three groups, and their associations with depressive symptoms and suicidality in HD mutation carriers, were analysed using multilevel regression analyses. There were no differences in parameters of HPA axis activity between mutation carriers and controls, whereas pre-motor symptomatic mutation carriers had a significantly higher area under the curve to the increase (AUC_i) compared to motor symptomatic mutation carriers. In the entire HD cohort, HPA axis activity was not associated with depressive symptoms or suicidality. After stratifying mutation carriers into pre-motor, early and advanced disease stages, β values differed between these groups. Remarkably, a higher AUC_i was significantly associated with depressive symptoms in pre-motor and early disease stage mutation carriers, with a reverse non-significant association in advanced disease stage mutation carriers. The lower AUC_i in motor symptomatic mutation carriers and the varying associations with depressive symptoms and suicidality in pre-motor, early and advanced disease stages could possibly be explained by exhaustion of the HPA axis after prolonged stress-induced HPA axis hyperactivity and deserves further longitudinal study.

Introduction

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease caused by a CAG expansion in the HTT gene on chromosome 4. The disease is characterised by motor symptoms, cognitive decline, behavioural symptoms and psychiatric disorders.[1]

Depression is a common psychiatric disorder in HD,[2] which can precede the onset of motor abnormalities.[3] The prevalence of a depressed mood is reported to range from 33% to 69%, depending on the disease stage and assessment method used.[2] The presence of a depressed mood in HD is an important predictor of suicidal ideation[4;5] and suicidal behaviour.[5,6] Previous studies showed that completed suicide rates among HD mutation carriers are four- to eight-fold higher compared to the general population,[7,8] with increased suicidality prevalences (including both suicidal ideation and attempts) of up to 20% in the previous month in both pre-motor and motor symptomatic mutation carriers.[5] Although some of the psychiatric symptoms in HD might be attributed to environmental factors, pathophysiological mechanisms may also be involved, such as dysfunction in the caudate nucleus,[2;9] which is one of the brain structures with prominent cell loss in HD.[1]

In non-HD populations, the presence of depression and suicidality has also been associated with disturbed functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Although several studies indicated hyperactivity of the HPA axis in depressed patients or in those developing depression compared to non-depressed controls,[10-15] other studies have reported normal[16;17] or even lower HPA axis activity in depressed patients or those developing depression compared to non-depressed controls.[18-20] These inconsistent results might partly be explained by the existence of a non-linear association between HPA axis activity and depression, which implies that both hyper- and hypoactivity of the HPA axis can be associated with depression.[21]

Also, an association between dysfunction of the HPA axis (most commonly dexamethasone non-suppression) and completed suicide has repeatedly been described, mainly in depressed patients.[22-24] A meta-analysis showed that non-suppressors of the dexamethasone suppression test (DST) are 4.7 times more likely to commit suicide than suppressors.[25] Evidence for a relationship between HPA axis functioning and suicidal ideation or attempted suicide is less consistent[22,24,26] (i.e. some studies reported hyperactivity of the HPA axis in those with suicidal ideation or attempts, whereas others found no association or reported a reverse association).[22;24;26-28]

Hypothalamic changes and hyperactivity of the HPA axis have been reported in HD mouse

models and in HD mutation carriers compared to controls.[29-38] Both alterations have been reported in early disease stages[30;34;37] and elevated salivary cortisol concentrations were found in pre-motor symptomatic HD mutation carriers compared to diagnosed HD patients.[39;40] Elevated cortisol concentrations in an early stage of HD could indicate that psychiatric symptoms in this stage are related to a perturbed HPA axis[30] and it has been suggested that HPA axis alterations may contribute to depressive symptoms in HD.[41] A recent study reported higher salivary morning cortisol concentrations in early disease stage mutation carriers with mild to severe depressive symptoms compared to non-depressed early disease stage mutation carriers,[40] whereas other studies found no significant relationship between HPA axis activity and depression scores,[30;42] the presence of a major depressive disorder (MDD) or dysthymia,[38] or a history of MDD.[31] So far, studies investigating the relationship between HPA axis functioning and psychiatric symptoms in HD were limited to the presence of depression and did not investigate suicidality. Furthermore, these studies were small (≤ 56 mutation carriers) and reported contradicting results.

In a previous cross-sectional study conducted by our group,[39] in contrast to earlier studies, no significant differences were found in overall parameters of HPA axis activity between HD mutation carriers and controls. For the present study, additional follow-up data was collected, and we aimed to compare HPA axis functioning, as measured by salivary cortisol concentrations at different time points during the day, between HD mutation carriers and controls at both baseline and follow-up measurements combined. We hypothesised that HPA axis activity would be elevated in both pre-motor and motor symptomatic mutation carriers compared to controls. We also aimed to assess the change in parameters of HPA axis activity between baseline and follow-up and expected to find a decrease in HPA axis functioning with disease progression. In addition, we aimed to investigate whether elevated HPA axis activity was associated with severity of depressive symptoms and suicidality within the cohort of HD mutation carriers and we expected to find higher depressive symptom and suicidality scores in mutation carriers with elevated parameters of HPA axis activity.

Materials and methods

Participants

Between May 2004 and August 2006, 152 HD mutation carriers were recruited for participation through the outpatient departments of Neurology and Clinical Genetics of the Leiden University Medical Center (LUMC) and a regional nursing home specialised in care for HD patients. In addition, 56 relatives, who initially had a 50% risk for the mutation but tested negative for the

HD mutation, were recruited as a control group. The study design and recruitment procedure have been described in detail elsewhere.[43]

A second measurement was made between 2006 and 2008; in this period, 128 mutation carriers and 42 non-carriers continued to participate. A third measurement was made between 2009 and 2011, which included 94 mutation carriers and 32 non-carriers. Biomarker measurements, including cortisol measurements at seven different time points during two consecutive days to assess HPA axis functioning, were made at the second and third measurements. Because several participants refused saliva collection or insufficient saliva was collected, only 97 mutation carriers and 33 controls could participate at the first cortisol measurement (2006–2008; baseline for this substudy) and 55 mutation carriers and 22 controls could participate at the second cortisol measurement (2009–2011; follow-up) (Figure 1).

Data from the first cortisol measurement were also used in a previous study investigating HPA axis functioning in HD compared to controls;[39] however, this latter study was limited to cross-sectional analyses with baseline data only. After the analyses for the previous study, additional data on baseline HPA axis activity were acquired in 13 mutation carriers and five controls and follow-up data on HPA axis activity were acquired in 55 mutation carriers and 22 controls. Also, the previous study did not investigate the change in parameters of HPA axis activity between baseline and follow-up and did not investigate the association between HPA axis functioning on the one hand and depressive symptoms and suicidality on the other. In addition, for the present study, all salivary cortisol samples used in the previous article were re-assayed using an improved re-standardised cortisol in saliva assay that guarantees accurate values in the 5–50 nmol/l range. Correlation coefficients between cortisol concentrations at the different times throughout the day, as determined by the former and the improved assay, ranged from 0.96 to 1.00. This improved assay was also used to assess the newly collected salivary cortisol samples.

The study was approved by the Medical Ethical Committee of the LUMC and all participants provided their written informed consent.

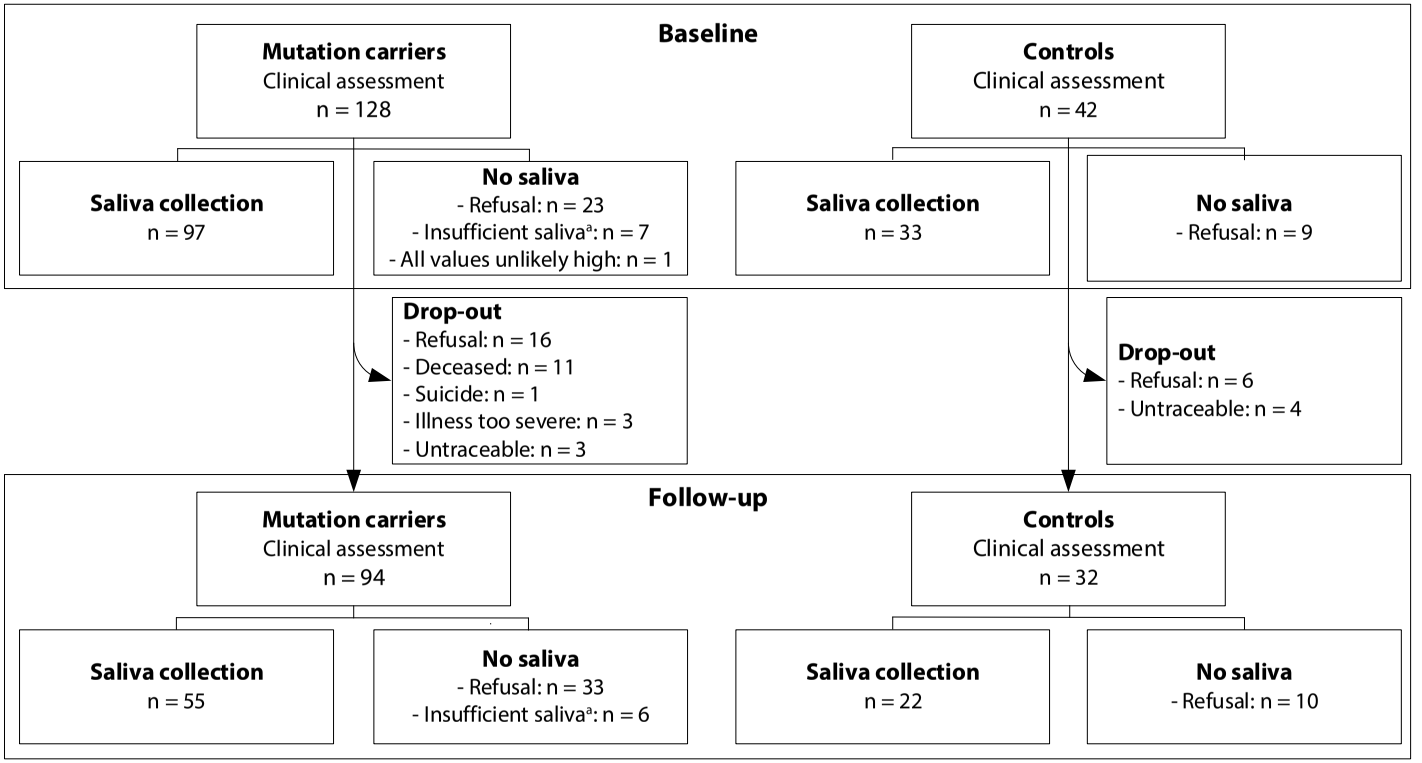

Figure 1. Flowchart of the study participants.

[a] Participants with > 4 missing cortisol concentrations (as a result of insufficient saliva collection) were excluded from the analyses.

Assessment of HPA axis functioning

HPA axis functioning was assessed by measuring cortisol concentrations in saliva samples. All participants received an oral and written instruction that asked them to collect saliva on two consecutive days. Instructions provided by the manufacturer were used. The participants had to place cotton wads from a saliva collection tube (Salivettes, catalogue no. 1.1534, Sarstedt AG & Co., Nümbrecht, Germany) in their mouth and chew until the cotton was saturated. To avoid contamination of the saliva with food or blood, participants were asked to refrain from eating, drinking or brushing their teeth before sampling. The participants were free to wake up on the basis of their own time schedule.

The day curve of cortisol concentrations was assessed by collecting the saliva on six different times throughout the day: at the time of awakening, 30 min after awakening, 45 min after awakening, 60 min after awakening, and at 22.00 and 23.00 h. In addition, the saliva cortisol concentrations after the DST were measured at the time of awakening on day two. Participants were also asked to record the date and time of collection. We assumed cortisol concentrations ≥ 100 nmol/l to be physiologically unlikely (e.g. as a result of bleeding gums) and these values were excluded. One or more cortisol values were excluded for this reason in two control participants (3.6%), two pre-motor symptomatic (4.1%) and five motor symptomatic mutation carriers (4.9%). When one to four cortisol values were missing, $\log_e$-transformed cortisol concentrations were imputed using multiple imputation (five times), in which other known characteristics of the participant were used: the remaining $\log_e$-transformed cortisol concentrations, time of awakening at day 1, time of awakening at day 2, season, sex, age, smoking, high alcohol use, body mass index (BMI), $\log_e$-transformed Total Functional Capacity (TFC) score, $\log_e$-transformed depressive symptom score, $\log_e$-transformed suicidality score, use of psychotropics, and time of measurement (baseline/follow-up). In total, from both baseline and follow-up combined, 63 of the 1449 (4.3%) cortisol values were imputed.

Two indicators of the cortisol awakening response (CAR), the area under the curve to the ground (AUC_g) and the area under the curve to the increase (AUC_i), were calculated using the first four time points, according to the trapezoid formula.[44] The mean evening cortisol was assessed by the mean of the two evening measurements on day 1, at 22.00 and 23.00 h. Physiologically, the DST results in a decrease in the morning cortisol concentration because of the inhibition of ACTH secretion. On the evening of day 1, the participants were instructed to orally take a low-dose dexamethasone tablet (0.5 mg). The dexamethasone suppression rate was determined by the cortisol concentration at time of awakening on day 1/the cortisol concentration at time of awakening on day 2 (post DST).

After collecting all seven saliva samples, participants returned their samples through the regular postal service. Upon arrival, saliva was harvested by centrifuging the Salivettes during 5 min at 10°C and 3309 g. Saliva was stored at -80 °C until analysis. Determination of cortisol in saliva was performed with a competitive electrochemoluminescent immunoassay using a Modular Analytics E170 immunoassay analyser (Roche Diagnostics, Mannheim, Germany). For the determination, a cortisol reagent (catalogue number 11875116 122/lot number 00168267) and calibrator (catalogue number 11875124 122/lot number 00169356: expiration date December 2013) were used.

The functional sensitivity for salivary cortisol is < 8.5 nmol/l, with a lower limit of detection of 0.5 nmol/l (source: Roche Diagnostics product insert). The internal quality control was monitored using home-made controls of saliva pools manufactured in an ISO-certified production facility (ISO 13485:2003). During the measurements, the mean ± SD was 8.04 ± 0.57 nmol/l for pool 1/20111281 (inter-run coefficient of variation [CV] = 7.11%; n = 28) and 20.70 ± 0.97 nmol/l (inter-run CV = 4.69%; n = 28) for pool 2/20111282.

Sociodemographic and clinical characteristics

Information on sociodemographic and clinical characteristics (e.g. height and weight, current smoking, high alcohol consumption [defined as drinking > 14 standardised units of alcohol a week] and medication use) was collected using a standardised interview. The estimated duration of disease was calculated by the current age minus the estimated age of onset according to the equation of Vassos et al.[45] Global daily functioning and disease stage[46] were assessed by the TFC scale[47] of the Unified Huntington's Disease Rating Scale (UHDRS),[48] and motor functioning was assessed by a trained neurologist using the motor scale of the UHDRS.[48] The confidence level (CL) of the UHDRS motor scale[48] was used to characterise mutation carriers as pre-motor (CL 0–1 points) or motor symptomatic (CL 2-4 points).

Assessment of depressive symptoms and suicidality

Depressive symptoms and suicidality were assessed using the Dutch version of the Problem Behaviours Assessment (PBA). The PBA is a semi-structured interview designed to assess the frequency and severity of 36 common behavioural symptoms in HD.[49;50] Total scores for the separate items of the PBA are computed by multiplying severity (range 0–4 points) and frequency (range 0–4 points) scores (range 0–16 points). Depressive symptoms in the previous month were assessed using the depressive symptoms cluster that was previously reported.[50] This depression subscale (range 0-80 points) consists of five items: 'depressed mood', 'depressive cognitions', 'anxiety', 'tension', and 'suicidal ideation'.[50] In addition, the 'suicidal ideation' item of the PBA (range 0–16 points) was used to assess the presence of suicidal ideation and suicide

attempts in the month preceding the interview.

Statistical analysis

Because of their positively skewed distributions, parameters of HPA axis activity, depressive symptom scores and suicidality scores were $\log_e$-transformed before being analysed. Data are presented as n (%), mean (± SD) or geometric back-transformed mean (i.e. back-transforming the mean of logarithmic values) (95% confidence intervals [CI]) when appropriate.

Sociodemographic and clinical characteristics of controls and pre-motor and motor symptomatic mutation carriers at baseline were compared using chi-squared tests for categorical data and one-way between-groups analysis of variance (ANOVA) for continuous data. Post-hoc comparisons were made using a chi-squared test for categorical data and Tukey's honestly significant difference test for continuous data.

Parameters of HPA axis activity of controls and pre-motor and motor symptomatic mutation carriers, both at baseline and follow-up combined, were compared using multilevel regression analyses (i.e. linear mixed models), with additional adjustment for potential confounders (sex, age, and time of measurement [baseline/follow-up]). An unstructured covariance matrix was used to account for the repeated measurements within a participant. Additionally, changes in the parameters of HPA axis activity between baseline and follow-up were compared between controls and pre-motor and motor symptomatic mutation carriers, using ANOVA. Analysis of covariance (ANCOVA) was used to adjust for sex and age.

The associations between continuous parameters of HPA axis activity on the one hand, and continuous depressive symptom and suicidality scores on the other, in HD mutation carriers at both baseline and follow-up combined were analysed using multilevel regression analyses with an unstructured covariance matrix. Analyses were adjusted for the potential confounders sex, age, season, smoking and time of measurement (baseline/follow-up). Because a previous study reported a relationship between HPA axis functioning and depression only in early symptomatic HD mutation carriers,[40] we stratified the mutation carriers into pre-motor symptomatic, early disease stage (motor symptomatic and disease stage 1 or 2)[46] and advanced disease stage (motor symptomatic and disease stage 3–5)[46] and repeated the analyses to explore whether the associations varied between these different disease stages.

In additional sensitivity analyses, BMI and alcohol use were added to the models of the original analyses. Also, analyses were restricted to those who woke up at, or before, 09.00 h and to those who reported the time of saliva collection and did not violate the prescribed time for

saliva collection by more than 5 min. For the DST, we considered participants with more than 60 min between time of awakening on day 1 and time of awakening on day 2 as time protocol violators.

For illustrative purposes only, scores on the outcome measures (depressive symptom and suicidality scores) were categorised into quartiles of parameters of HPA axis activity in the tables and figures, whereas statistical tests were solely based on the appropriate continuous data. $P < 0.05$ (two-tailed) was considered statistically significant. SPSS, version 20.0 (IBM Corp., Armonk, NY, USA) was used for the analyses.

Results

Baseline characteristics of pre-motor and motor symptomatic mutation carriers versus controls
At baseline, the study population consisted of 33 controls, 30 pre-motor symptomatic mutation carriers and 67 motor symptomatic mutation carriers (Table 1). Motor symptomatic mutation carriers were significantly older compared to pre-motor symptomatic mutation carriers and controls, and also had lower TFC scores. In addition, the use of psychotropic medication showed a significant difference between the three groups. Depressive symptom scores were higher in both pre-motor and motor symptomatic HD mutation carriers compared to controls. Also, suicidality scores in pre-motor and motor symptomatic mutation carriers were higher compared to controls; however, these differences were not significant (Table 1).

Table 1. Sociodemographic and clinical characteristics of controls and pre-motor and motor symptomatic Huntington's disease mutation carriers at baseline.

	Controls (n = 33)	**Pre-motor symptomatic** (n = 30)	**Motor symptomatic** (n = 67)	**p-value**[a]
Sociodemographic characteristics				
Male gender	16 (48.5)	13 (43.3)	33 (49.3)	0.86
Age (years)	43.7 ± 11.2[A]	43.4 ± 10.9[A]	52.7 ± 10.1[B]	<0.001
Body mass index (kg/m^2)	25.5 ± 4.45	25.9 ± 6.08	25.7 ± 4.64	0.95
Smoking (yes/no)	7 (21.2)	8 (26.7)	15 (23.8)	0.88
High alcohol consumption	0 (0)	4 (13.3)	6 (9.0)	0.12
Clinical characteristics				
CAG repeats (number)	21.5 ± 4.56[A]	42.1 ± 2.33[B]	44.4 ± 3.12[C]	<0.001
Estimated disease duration (years)	NA	-6.67 ± 9.14[A]	8.28 ± 8.20[B]	<0.001
Motor score (points)[b]	NA	3.27 (2.28 – 4.34)[A]	30.4 (24.7 – 37.0)[B]	<0.001
Total Functional Capacity score (points)[b]	12.8 (12.6 – 13.0)[A]	11.6 (10.7 – 12.5)[A]	6.17 (5.08 – 7.34)[B]	<0.001
Use of psychotropics	1 (3.0)[A]	6 (20.0)[B]	42 (62.7)[C]	<0.001
Neuropsychiatric characteristics				
PBA depressive symptom score (points)[b]	3.13 (1.67 – 4.78)[A]	7.23 (3.91 – 11.3)	7.19 (4.91 – 9.81)[B]	0.04
PBA suicidality[b]	0.10 (0.00 – 0.31)	0.34 (0.00 – 0.74)	0.65 (0.17 – 1.14)	0.22

Data are presented as n (%), mean ± SD, or geometric mean values (i.e. back-transforming the mean of logarithmic values) (95% confidence interval). PBA denotees Problem Behaviours Assessment; NA, not applicable.

[a] P-values determined using a chi-squared test for categorical data and one-way between-groups ANOVA (or t-test when comparing only two groups) for continuous data. Post-hoc comparisons were made using a chi-squared test for categorical data and a Tukey's honestly significant difference test for continuous variables. Values in the same row with different superscript letters (uppercase) are significantly different (p-value < 0.05).

[b] Because of its skewed distribution, these variables were $\log_e$-transformed before the analyses.

Parameters of HPA axis activity in HD mutation carriers versus controls

Comparison of the parameters of HPA axis activity between controls and pre-motor symptomatic and motor symptomatic mutation carriers, at both baseline and follow-up combined, showed that the only significant difference was a higher AUC_i in pre-motor symptomatic mutation carriers compared to motor symptomatic mutation carriers (Table 2a); this difference remained significant after adjustment for sex and age. Although the AUC_g was also higher in pre-motor symptomatic mutation carriers compared to motor symptomatic mutation carriers and controls, this difference was not significant. Also, the mean evening cortisol and the cortisol suppression ratio showed no significant difference between controls and pre-motor and motor symptomatic mutation carriers, in both the crude and adjusted models (Figure 2 and Table 2a).

The largest change in parameters of HPA axis activity from baseline to follow-up was observed for the AUC_i (> 9% decrease in all groups). However, the changes in parameters of HPA axis activity between baseline and follow-up showed no significant difference between controls and pre-motor and motor symptomatic mutation carriers, in both the crude and adjusted comparisons for all parameters (Table 2b).

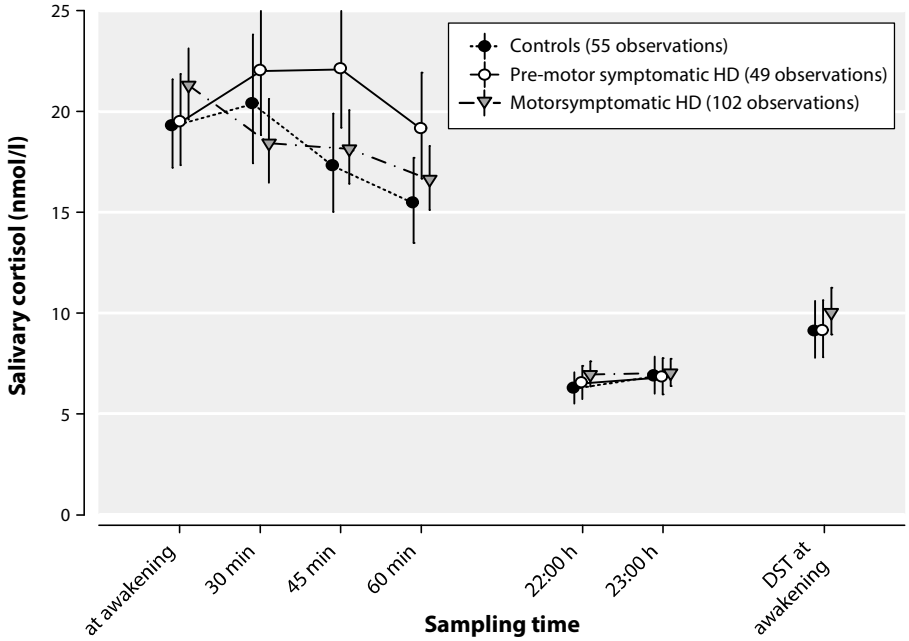

Figure 2.

Geometric mean cortisol concentrations (i.e. back-transforming the mean of logarithmic values) (95% confidence interval) at the different sampling times for controls and pre-motor and motor symptomatic HD mutation carriers at both baseline and follow-up combined. For one observation, a motor assessment was missing, which resulted in 55 control and 151 mutation carrier observations.

Table 2a. Parameters of hypothalamic-pituitary-adrenal (HPA) axis activity of controls and pre-motor and motor symptomatic Huntington's disease mutation carriers, at baseline and follow-up combined.

	Controls (observations = 55)[a]	**Pre-motor symptomatic** (observations = 49)[a]	**Motor symptomatic** (observations = 102)[a]	**p-value**[b]	**Adjusted p-value**[c]
Parameters of HPA axis activity[d]					
AUC to the ground (nmol/l)	1149 (1028; 1284)	1306 (1168; 1461)	1181 (1091; 1279)	0.22	0.20
AUC to the increase (nmol/l)	-17.6 (-138; 104)	123 (-2.57; 250)[A]	-139 (-233; -43.7)[B]	0.004	0.002
Mean evening (nmol/l)	6.62 (5.88; 7.47)	6.88 (6.11; 7.75)	7.09 (6.52; 7.72)	0.65	0.76
Cortisol suppression ratio	2.12 (1.85; 2.43)	2.17 (1.88; 2.50)	2.08 (1.87; 2.31)	0.85	0.95

Table 2b. Change in parameters of hypothalamic-pituitary-adrenal (HPA) axis activity of controls and pre-motor and motor symptomatic Huntington's disease mutation carriers, from baseline to follow-up.

	Controls (n = 21)	**Pre-motor symptomatic** (n = 20)	**Motor symptomatic** (n = 32)	**p-value**[e]	**Adjusted p-value**[f]
Delta measurements (values t2–t1)					
Delta AUC to the ground (nmol/l)	-2.63 (-210; 205)	-21.9 (-383; 340)	7.72 (-111; 126)	0.97	0.96
Delta AUC to the increase (nmol/l)	-155 (-300; -11.1)	-11.3 (-333; 310)	-41.3 (-215; 132)	0.59	0.54
Delta mean evening cortisol (nmol/l)	-1.14 (-3.65; 1.36)	0.35 (-1.46; 2.15)	0.08 (-0.65; 0.80)	0.40	0.48
Delta cortisol suppression ratio	2.64 (-2.58; 7.86)	-0.14 (-0.70; 0.41)	-0.01 (-0.44; 0.41)	0.25	0.11

AUC denotes area under the curve. Data are presented as geometric mean values (i.e. back-transforming the mean of logarithmic values) (95% CI) in table 2a and as mean (95% CI) in table 2b. Values in the same row with different superscript letters (uppercase) are significantly different (p-value < 0.05).

[a] For one observation, a motor assessment was missing which resulted in 55 control and 151 mutation carrier observations in these analyses.

[b] P-values by multilevel regression analyses.

[c] P-values adjusted for sex, age, and measurement (baseline/follow-up) calculated with multilevel regression analyses.

[d] Because of its skewed distribution, these measures were $\log_e$-transformed before the analyses.

[e] P-values by analysis of variance (ANOVA).

[f] P-values adjusted for sex and age calculated with analysis of covariance (ANCOVA).

Associations between parameters of HPA axis activity and depressive symptoms and suicidality
Analyses of the associations between parameters of HPA axis activity and PBA depressive symptom score (Table 3) showed that the strongest but non-significant association was with the AUC_i. No associations were found between the AUC_g, mean evening cortisol and cortisol suppression ratio (CSR) on the one hand and depressive symptom score on the other. After adjustment for potential confounders, the β values remained similar or became smaller compared to those in the crude model (Figure 3 and Table 3).

Analyses of the associations between parameters of HPA axis activity and suicidality showed that the AUC_g, AUC_i, mean evening cortisol and the CSR were not associated with suicidality score, either in the crude or in the adjusted model (Figure 3 and Table 3).

Associations in pre-motor symptomatic, early and advanced disease stages
When exploring associations separately in pre-motor symptomatic (49 observations), early disease stage (48 observations) and advanced disease stage (53 observations) mutation carriers, both the crude and adjusted β values differed for all parameters of HPA axis activity between the three groups (data not shown). For the depressive symptom score, the difference between the adjusted β values was largest for the AUC_i. A higher AUC_i was associated with depressive symptoms in both pre-motor symptomatic ($\beta_{adjusted}$ = 0.27, 95% CI = 0.04 to 0.51) and early disease stage mutation carriers ($\beta_{adjusted}$ = 0.44, 95% CI = 0.21 to 0.67), whereas a reverse association was found in advanced disease stage mutation carriers ($\beta_{adjusted}$ = -0.09, 95% CI = -0.38 to 0.21). Regarding suicidality, the difference between adjusted β values was largest for the CSR. A lower CSR was associated with suicidality in the pre-motor symptomatic group ($\beta_{adjusted}$ = -0.19, 95% CI = -0.43 to 0.06). This association was weaker in the early disease stage group ($\beta_{adjusted}$ = -0.04, 95% CI = -0.36 to 0.28) and was reversed in the advanced disease stage group ($\beta_{adjusted}$ = 0.15, 95% CI = -0.23 to 0.53).

Sensitivity analyses
For both the depressive symptom and suicidality analyses in the entire group of HD mutation carriers, additional adjustments for BMI and alcohol use resulted in comparable or smaller β values compared to the original adjusted models, which differed no more than 0.05 points from those of the original analyses. Also, when restricting the analyses to those mutation carriers who woke up at, or before, 09.00 h, the crude and adjusted β values for both depressive symptoms and suicidality differed no more than 0.05 points from those of the original analyses. When time protocol violators were excluded from the analyses, the crude and adjusted β values for both depressive symptoms and suicidality differed no more than 0.04 points from those of the original analyses (data not shown).

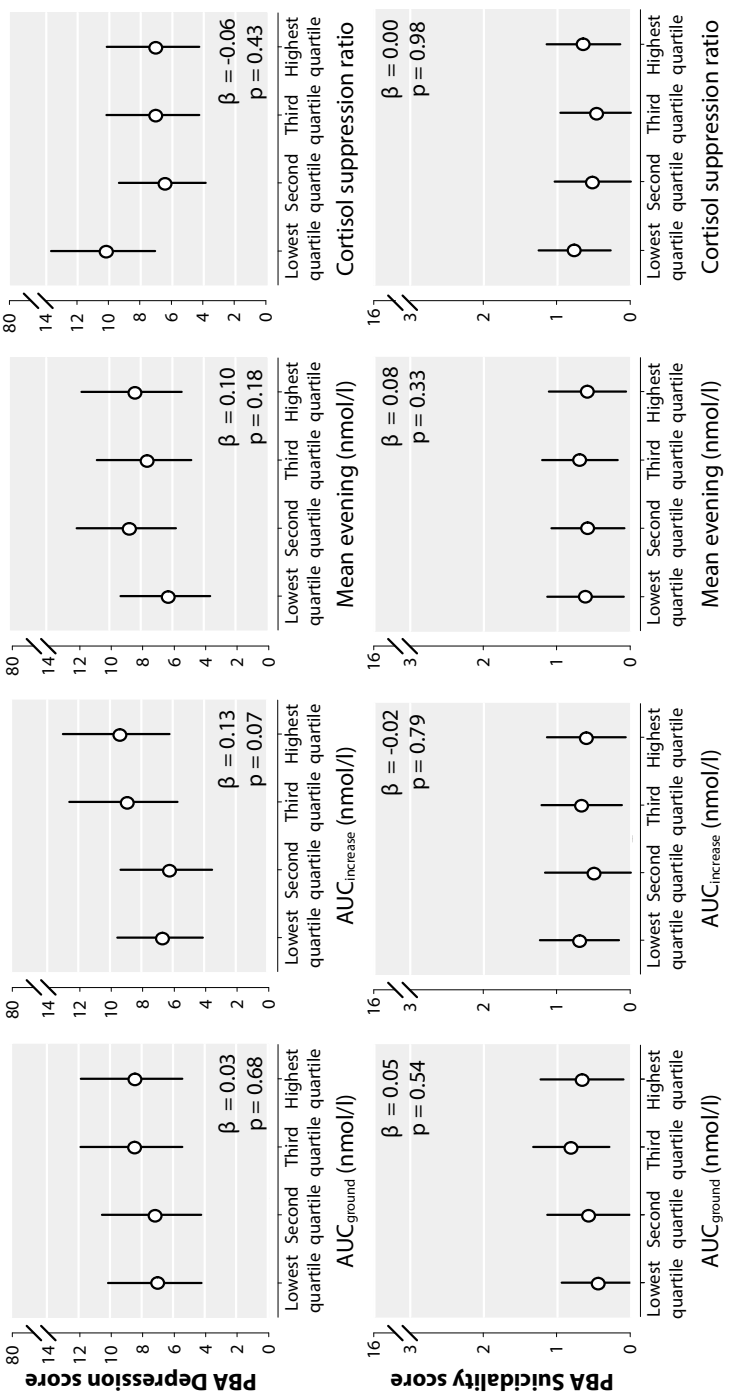

Figure 3.
Geometric mean Problem Behaviours Assessment (PBA) depressive symptom and suicidality scores (i.e. back-transforming the mean of logarithmic values) (95% confidence interval) are given in different hypothalamic-pituitary-adrenal (HPA) axis parameter quartiles for illustrative purposes only. Unadjusted β values and p-values by multilevel regression analyses (log_e-transformed parameters of HPA axis activity as continuous determinant, log_e-transformed depressive symptom or suicidality score as continuous outcome). Results are based on 151 observations (one missing observation on the PBA), with both baseline and follow-up combined. AUC denotes area under the curve.

Table 3. Associations between parameters of hypothalamic-pituitary-adrenal (HPA) axis activity on the one hand, and depressive symptom and suicidality score on the other, at both baseline and follow-up combined.

	Lowest quartile HPA axis parameter	Second quartile HPA axis parameter	Third quartile HPA axis parameter	Highest quartile HPA axis parameter	β (95% CI)	p-value	Adjusted β (95% CI)[a]	Adjusted p-value[a]
AUC_g[b]	(Observ. = 39)	(Observ. = 37)	(Observ. = 37)	(Observ. = 38)				
Range	337; 960	961; 1217	1218; 1567	1568; 5014				
Depression score	6.90 (4.20 – 10.1)	7.08 (4.22 – 10.5)	8.37 (5.42 – 11.9)	8.35 (5.41 – 11.8)	0.03 (-0.12; 0.18)	0.68	0.00 (-0.15; 0.16)	0.97
Suicidality score	0.43 (0.00 – 0.95)	0.56 (0.02 – 1.14)	0.80 (0.30 – 1.33)	0.65 (0.10 – 1.23)	0.05 (-0.11; 0.20)	0.54	0.06 (-0.11; 0.22)	0.49
AUC_i[b]	(Observ. = 38)	(Observ. = 39)	(Observ. = 37)	(Observ. = 37)				
Range	-2136; -281	-280; -63.8	-63.7; 253	254; 1384				
Depression score	6.59 (4.10; 9.52)	6.15 (3.50 – 9.32)	8.82 (5.69 – 12.6)	9.30 (6.20 – 13.0)	0.13 (-0.01; 0.27)	0.07	0.14 (-0.01; 0.28)	0.06
Suicidality score	0.69 (0.17 – 1.24)	0.49 (0.00 – 1.17)	0.66 (0.13 – 1.22)	0.59 (0.07 – 1.14)	-0.02 (-0.17; 0.13)	0.79	-0.01 (-0.17; 0.15)	0.91
Mean evening[b]	(Observ. = 37)	(Observ. = 38)	(Observ. = 38)	(Observ. = 38)				
Range	2.94; 5.29	5.30; 6.55	6.56; 8.63	8.64; 27.1				
Depression score	6.19 (3.60 – 9.29)	8.65 (5.77 – 12.1)	7.53 (4.79 – 10.8)	8.31 (5.41 – 11.8)	0.10 (-0.05; 0.26)	0.18	0.05 (-0.13; 0.22)	0.60
Suicidality score	0.61 (0.10 – 1.14)	0.58 (0.09 – 1.08)	0.68 (0.18 – 1.21)	0.58 (0.07 – 1.12)	0.08 (-0.08; 0.23)	0.33	0.03 (-0.15; 0.22)	0.71
CSR[b]	(Observ. = 38)	(Observ. = 38)	(Observ. = 38)	(Observ. = 37)				
Range	0.42; 1.60	1.61; 2.07	2.08; 2.85	2.86; 7.77				
Depression score	10.1 (7.10 – 13.7)	6.40 (3.89 – 9.38)	6.98 (4.28 – 10.2)	6.97 (4.29 – 10.2)	-0.06 (-0.20; 0.09)	0.43	-0.06 (-0.20; 0.09)	0.43
Suicidality score	0.76 (0.26 – 1.30)	0.56 (0.07 – 1.06)	0.39 (0.00 – 0.90)	0.72 (0.18 – 1.29)	0.00 (-0.17; 0.17)	0.98	0.02 (-0.15; 0.18)	0.83

AUC_g denotes area under the curve to the ground; AUC_i, area under the curve to the increase; CSR, cortisol suppression ratio; observ., observations.
Geometric mean Problem Behaviours Assessment (PBA) depressive symptom and suicidality scores (i.e. back-transforming the mean of logarithmic values) (95%

confidence interval [CI]) are given in different HPA axis parameter quartiles for illustrative purposes only. β values (95% CI) and p-values by multilevel regression analyses (log_e-transformed parameters of HPA axis activity as continuous determinant, log_e-transformed depressive symptom or suicidality score as continuous outcome).
Crude analyses: 151 observations (one missing on the PBA), adjusted analyses: 147 observations (four additional missings on smoking status).
[a] Adjusted for: sex, age, season, smoking, measurement (baseline/follow-up).
[b] Because of their skewed distribution, the PBA depressive symptom score, PBA suicidality score, and parameters of HPA axis activity were log_e-transformed before the analyses.

Discussion

The present study examined HPA axis activity in HD and revealed a significantly higher AUC_i in pre-motor symptomatic mutation carriers compared to motor symptomatic mutation carriers. By contrast to our hypothesis, we found no differences in HPA axis activity between both pre-motor and motor symptomatic mutation carriers and controls. Also, no overall statistically significant or clinically relevant associations were found between parameters of HPA axis activity on the one hand, and depressive symptoms or suicidality on the other. Nevertheless, when the population was stratified in pre-motor symptomatic, early and advanced disease stages, the associations varied between the different stages, and the AUC_i was significantly associated with depressive symptoms in mutation carriers from both pre-motor symptomatic and early disease stages.

By contrast to previous studies,[29-33;38] we found no hyperactivity of the HPA axis in HD mutation carriers compared to controls. However, the previous studies differed from our study by including controls who did not grow up in a HD family.[29-31;33;38] There is increasing evidence that, as a result of epigenetic mechanisms, an unfavourable environment during childhood can influence important genes in stress regulation that can affect HPA axis functioning.[22;23;51] The stress of growing up in an HD family, and the fear of being an HD mutation carrier, was most probably also experienced by our control group, which may explain why, compared to non-depressed controls from the general Dutch population,[10] the salivary cortisol concentrations of the controls in the present study were higher at every measured time point (on average 2.65 nmol/l higher).

In addition, these contrasting studies assessed HPA axis functioning by measuring cortisol in serum,[30-33] urine,[29] or cerebrospinal fluid (CSF),[38] whereas the present study, as well as another study that found no differences in HPA axis activity between mutation carriers and controls,[40] assessed HPA axis functioning by measuring salivary cortisol. Most of these measures only indirectly assess central HPA axis functioning and all reflect partially different information regarding its activity, which can lead to different results.[52]

When comparing HPA axis activity within HD mutation carriers, we found a lower CAR in motor symptomatic mutation carriers compared to pre-motor symptomatic mutation carriers, which is in line with previous results from this cohort,[39] and with another study reporting higher morning salivary cortisol concentrations in pre-diagnosed mutation carriers compared to early HD.[40] Long-lasting periods of elevated HPA axis activity in the earlier phases of HD may lead to reduced HPA axis activity[53] in later phases as a result of volume reduction or impaired

functioning of the hippocampus,[26] resulting in a blunted CAR.[21]

The elevated activity preceding down-regulation or exhaustion of the HPA axis in HD might be explained by loss of HPA axis feedback mechanisms as a result of brain pathology, such as hypothalamic alterations, which occur before clinical diagnosis.[34;37] In addition, a loss of GABAergic neurons can result in disinhibition of the HPA axis,[31] and the experience of different kinds of stress because of the presence of HD in their family might have resulted in elevated morning cortisol concentrations. The hypothesis of reduced HPA axis activity after prolonged exposure to stress and elevated parameters of HPA axis activity was also proposed in a depression and anxiety population.[21] Within the HD population, this hypothesis is supported by a postmortem study reporting decreased corticotropin-releasing hormone (CRH) immunoreactivity in the striatum of symptomatic HD patients.[54] Furthermore, the finding that pre-motor symptomatic mutation carriers showed no decrease in parameters of HPA axis activity over time does not support this hypothesis. However, the pre-motor symptomatic group is a heterogeneous population with varying estimated times until motor onset and the individual changes in the CAR from baseline to follow-up differed substantially.

Overall, we found no statistical significant or clinically relevant associations between HPA axis activity and depressive symptoms which is in line with previous HD studies that investigated HPA axis functioning in CSF,[38] blood,[30;31;42] and saliva.[40] However, after stratifying the population of this latter study according to disease stage, salivary morning cortisol concentrations were found to be higher in early HD mutation carriers with mild to severe depressive symptoms compared to non-depressed early HD mutation carriers;[40] this finding is in line with the results of our subgroup analyses. It is possible that, in different stages of HD, disturbed HPA axis functioning may have a different influence on the complex aetiology of depression, for which several mechanisms, both neuropathological and environmental, have been proposed.[2;9]

Although these are only exploratory analyses in small subgroups, the subgroup analyses for the AUC_i do support the hypothesis of hyperactivation of the HPA axis in depressed mutation carriers in earlier disease stages, with exhaustion or down-regulation of the HPA axis after a longer period of stress-induced HPA axis hyperactivity,[21] as indicated by the reverse association in advanced stage motor symptomatic mutation carriers. Two other previous small HD studies that only included early symptomatic mutation carriers ($n = 8$[30] and $n = 56$)[38] did find higher HPA axis activity in depressed mutation carriers, although these associations were not significant. This might be the result of a lack of power, or because one of these studies focused on mean 24-h plasma cortisol concentrations[30] and the other on the afternoon CSF CRH concentration,[38] whereas both in HD and non-HD studies, the CAR was most frequently

associated with depression.[10;11;40]

This is the first study to investigate the relationship between HPA axis activity and suicidality in HD, finding no associations in the entire group of HD mutation carriers. First, the overall null finding might be explained by different associations in different disease stages, especially for the CSR, which was also most commonly associated with suicidality in non-HD populations.[22-24] In addition, previous non-HD studies showed that the relationship between HPA axis and suicidality is most consistent for completed suicide, whereas the results of studies investigating suicide attempts or suicidal ideation are less consistent[22;24;26-28] and might be dependent on the suicidality severity.[26] Because suicidal ideation, attempts, and completed suicides are separate phenotypes with probably only partly shared aetiology,[51] the relatively mild suicidality scores and lack of completed suicides in this cohort might also explain the lack of association in this HD population.

Furthermore, inconsistencies in results from non-HD studies on the association between HPA axis functioning and suicidality have been attributed to the presence of a deleterious childhood environment in study participants,[22;23;51] with early-life stress being a possible confounder in this relationship. It is possible that HD mutation carriers, regardless of whether they do or do not become suicidal, experienced some form of early-life stress. Unfortunately, we did not assess the extent of early-life stress experienced in our study population.

Several limitations of the present study limit causal inference from our results. First, because of the limited number of incident depression and suicidality cases, we focused on cross-sectional analyses. Because timing is crucial in the investigation of HPA axis functioning and the direction of possible relationships cannot be determined in cross-sectional studies, longitudinal studies are needed to investigate changes in the HPA axis preceding the onset of behavioural symptoms. Second, many different mechanisms can result in either hyper- or hypoactivity of the HPA axis, some of which might be associated with depression or suicidality, whereas others might not. This lack of consistency makes it impossible to disentangle the complex causal relationship between HPA axis functioning and behavioural symptoms in an observational study.[55] Third, unknown and unmeasured negative confounding may have prevented us from finding potentially causal associations. Furthermore, the stratified analyses were only explorative in relatively small groups. Another limitation of the present study is the use of peripheral markers that do not necessarily reflect all regulatory processes of central activation of the HPA axis. The sophisticated physical feedback regulation systems might keep peripheral cortisol concentrations at normal levels whereas hormones in the brain regulating peripheral cortisol concentrations might be dysregulated. Also, the relatively mild depressive

symptom and suicidality scores in this population may limit external generalisability of our findings because the associations in more severely affected populations might be different.

Previously, antiglucocorticoid therapy was suggested, for example, for the treatment of behavioural symptoms in HD.[30] However, because of the limitations of this observational study, only a randomised clinical trial with antiglucocorticoid therapy could lead to causal conclusions on the effectiveness of such therapy. However, in line with previous HD studies, the present study, with several strong aspects such as a relatively large HD population, the assessment of multiple parameters of HPA axis activity and adjustment for various confounders, found no evidence for an association between HPA axis activity and behavioural symptoms in HD. The associations between HPA axis activity and depression and suicidality appear to vary in different HD stages, which might be explained by the exhaustion of the HPA axis after prolonged stress-induced HPA axis hyperactivity. Exhaustion of the HPA axis might also explain the diminished concentrations of morning cortisol in motor symptomatic mutation carriers. However, further longitudinal studies are needed to determine the course of HPA axis functioning during disease progression.

Acknowledgments

The authors thank N.A. Aziz, S.J.A. Bogaard and Y.A.M. Grimbergen (neurologists) for performing the UHDRS motor score.

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