Huntington's disease : hypothalamic, endocrine and metabolic aspects

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Chapter 8

Growth hormone and ghrelin secretion are associated with clinical severity in Huntington's disease

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Eur J Neurol (accepted for publication)

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ABSTRACT

Background: Huntington's disease (HD) is a fatal hereditary neurodegenerative disorder caused by an increased CAG repeat size in the *huntingtin* gene. Apart from neurological impairment, the disease is also accompanied by progressive weight loss, abnormalities in glucose homeostasis and a higher prevalence of diabetes mellitus, which may partly be caused by disturbed growth hormone (GH) and ghrelin secretion. Therefore, we aimed to perform a detailed analysis of GH and ghrelin secretion in HD patients in relation to clinical signs and symptoms. *Methods:* In nine early-stage, medication-free HD patients and nine age-, sex- and body mass index (BMI)-matched controls, we measured serum GH levels every 10 min for 24 h and assessed ghrelin response to food intake. Multi-parameter auto-deconvolution and approximate entropy analysis were applied to quantify basal, pulsatile and total GH secretion rates as well as the regularity of GH secretion. *Results:* We found no significant differences in GH and ghrelin secretion characteristics between HD patients and controls (total GH secretion: 137 ± 36 *vs.* 181 ± 43 mU/L/24h, respectively; $p=0.439$). However, in HD patients, both GH secretion and its irregularity as well as the degree of postprandial ghrelin suppression significantly increased with worsening motor and functional impairment (all $p<0.05$). Moreover, postprandial ghrelin suppression also increased with decreasing body weight and higher CAG repeat number (both $p<0.05$). *Conclusions:* These findings suggest changes in the regulation of GH and ghrelin secretion dynamics in early stage HD patients that could become more prominent in the later stages of the disease.

Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat size in the gene encoding the protein huntingtin.[1] The disease is characterized by motor disturbances, cognitive deterioration, and psychiatric and behavioural problems.[1] Progressive weight loss and muscle wasting are also hallmarks of the disease, both in HD patients [2-6] and several transgenic mouse models of the disease.[7,8] Moreover, profound abnormalities in glucose homeostasis as well as a higher prevalence of diabetes mellitus have been reported in HD patients, which are also evident in the transgenic models.[9,10] The cause of these peripheral signs is largely unknown, although hypothalamic dysfunction, and subsequent endocrine alterations may be involved.[2,11]

The somatotropic axis, which plays an essential role in body energy homeostasis, is among the hypothalamic-endocrine axes that could be affected in HD.[2,11] Exaggerated growth hormone (GH) responses have been observed following administration of L-dopa[12], apomorphine[13], bromocriptine[14], arginine[15,16], insulin[17-19] and muscimole[13], whereas paradoxical GH responses have been reported after glucose[12,20-22], L-dopa[20] or bromocriptine[20,23] administration. Increased mean serum GH concentrations have also been found in HD patients [24,25]. However, others have not been able to detect abnormalities in GH release.[21,26] These discrepancies are likely due to the use of a few baseline measurements of GH levels or long blood sampling intervals which are not adequate to assess either the pulsatile nature of GH secretion or its total daily production.[27]

Elevated plasma levels of ghrelin, an orexigenic hormone of gastric origin which stimulates GH release,[28] have also been reported in HD patients.[29,30] Ghrelin, like GH, is an endogenous regulator of energy homeostasis; ghrelin levels increase with fasting and fall postprandially, thereby signaling mealtime hunger and satiety to the brain.[28,31] However, the relation between GH and ghrelin secretion, as well as the association between postprandial ghrelin suppression and clinical phenotype has not been investigated in HD patients.

We postulated that GH secretion patterns are likely to be perturbed in HD patients. Moreover, as GH has profound effects on fat and muscle tissue, we hypothesized that the total daily GH production in HD patients would be associated with body weight, as well as fat and lean body mass. In addition, we postulated that ghrelin secretion and its relation to GH levels and clinical phenotype may be perturbed in HD patients. We tested these hypotheses by deconvolution analysis of 24 h serum GH concentration profiles and simultaneous assessment of plasma ghrelin response to food intake in both early stage HD patients and healthy matched controls.

SUBJECTS AND METHODS

Subjects

Nine early-stage HD patients and nine healthy control subjects, matched for age, sex, and body mass index (BMI), were enrolled in the study. Clinical details are summarized in **Table 1**. In the patient group, mutant CAG repeat size ranged between 41 and 50. The clinical diagnosis of HD was made by a neurologist specialized in movement disorders (R.A.C.R.). The Unified Huntington's Disease Rating Scale (UHDRS) was used to assess HD

Table 1. Characteristics of the study population

	HD patients†	Controls†	p-value‡
Male/female	6/3	6/3	-
Age [y]	47.1 (3.4)	48.6 (3.3)	0.764
BMI	24.1 (1.0)	24.3 (0.6)	0.876
Fat [%]	25.5 (2.4)	25.6 (2.4)	0.985
Lean body mass [kg]	57.3 (3.2)	56.2 (3.0)	0.800
Waist-to-hip ratio	0.89 (0.03)	0.94 (0.02)	0.147
Mutant CAG repeat size	44.4 (1.0)	-	-
Disease duration [y]	5.7 (1.1)	-	-
UHDRS motor score	22.2 (6.0)	-	-
TFC score	11.7 (0.7)	-	-
Functional Assessment	23.3 (0.7)	-	-
Independence score	94.4 (2.8)	-	-

†) Values are indicated as mean (SE).
‡) Differences between groups were assessed by unpaired t-tests.
Abbreviations: BMI = Body Mass Index; FAS = Functional Assessment; TFC = Total Functional Capacity; UHDRS = Unified Huntington's Disease Rating Scale.

symptoms and signs.[32] None of the subjects used medication, except one HD patient who discontinued paroxetine use three weeks prior to study. Subjects were eligible for participation after exclusion of hypertension, any known (history of) pituitary disease, recent intentional weight change (>3 kg weight gain or loss within the last 3 months), and any other chronic conditions except HD as assessed by clinical examination and routine laboratory tests. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the Leiden University Medical Center.

Clinical protocol

Subjects were admitted to the Clinical Research Center for 24 h blood sampling. Two women (one patient and one control) were postmenopausal, the other women were studied in the early follicular phase of their menstrual cycle. A cannula was inserted into an antecubital vein 45 min before the start of blood sampling at 1630 h. Blood samples were collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock that was attached to a 0.9% NaCl and heparin (1 U/ml) infusion (500 ml/24 h) to keep the cannula from clotting. Sampling was performed through a long line to prevent sleep disruption by investigative manipulations. During 24 h, blood was collected in serum tubes at 10-min intervals for GH measurements. From 0840 h to 1100 h — i.e. 20 min before to 2 h after the start of breakfast which was consumed between (but not including) 0900 and 0920 h — blood was also collected in separate EDTA tubes for the assessment of ghrelin levels. While blood in the serum tubes was allowed to clot, the EDTA tubes were immediately put on ice. Within 60 min of sampling, all tubes were centrifuged at 4000 rotations/min at 4 ºC for 20 min, and serum and plasma were stored at -80 ºC until assay. Three standardized meals were served at 0900, 1300, and 1900 h (Nutridrink, 1.5 kcal/ml, 1500–1800 kcal/d; macronutrient composition per 100 ml: protein, 5 g; fat, 6.5 g; carbohydrate, 17.9 g; Nutricia, Zoetermeer, The Netherlands). Subjects remained sedentary except for bathroom visits. Twenty-four hour urine was collected for the determination of creatinine and catecholamines concentrations. No daytime naps were allowed. Lights were switched off at 2300 h and, the next morning, subjects were awakened at 0730 h.

Body composition

Bioelectrical impedance analysis was used to assess lean body mass and fat percentage at 0800 h.

Assays

Serum GH was measured by time-resolved fluoroimmunoassay (DELFIA® hGH, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The detection limit of the assay was 0.03 mU/L, and the interassay variation ranged from 1.6 to 8.4%. Plasma total ghrelin levels were measured by radioimmunoassay (LINCO Research, St. Charles, MO, USA) with a detection limit of 93 pg/mL, and an interassay variation ranging from 14.7 to 17.8%. Samples from each patient and matched control were handled in the same run. Total serum insulin-like growth factor (IGF)-1 and insulin-like growth factor binding protein (IGFBP)-3 concentrations were also measured by radioimmunoassay as previously described.[33] Glycosylated hemoglobin (HbA1c) levels were measured with an high performance liquid chromatography (HPLC) system (Variant, Biomed, Hercules, CA, USA). Urine creatinine was measured by a fully automated P 800 Modular system (Roche, Almere, the Netherlands). Urinary epinephrine, norepinephrine and dopamine concentrations were assessed by HPLC with electron capture detection (ESTA-Coulochem, Chelmsford, MA, USA).

Calculations and statistics

Deconvolution analysis. A recently developed, fully automatic, multi-parameter deconvolution procedure, *AutoDecon,* was used to estimate various specific measures of secretion and serum disappearance rate of GH, considering all serum hormone concentrations and their dose-dependent intra-sample variance simultaneously.[34] The *AutoDecon* process is a statistically based algorithm to test the significance of hormone secretion events, obviating the subjective nature of previously used deconvolution methods.[34]Apart from the initial concentration and the basal secretion rate, which both were initialized to zero, the *AutoDecon* algorithm requires only two approximations of the parameter values that are to be estimated: (1) The standard deviation of the Gaussian-shaped secretion events (Secretion*SD*) which is generally initialized as half of the data-sampling interval, and (2) a starting value for the elimination parameter, or hormone half-life.[34] Thus, for 10-min sampled data, the Secretion*SD* was initialized to 5-min together with a starting value for the GH half-life of 16-min.[35] To account for intrinsic errors in the estimates of hormone secretion and clearance rates, the *AutoDecon* algorithm was then used to find the best fits for both parameters.[35] The following parameters of the GH time series were estimated: number of secretory bursts, secretory burst half-duration (duration at half-maximal amplitude), mean mass secreted per burst, hormone half-life, basal secretion rate, pulsatile secretion rate, and total secretion rate.

Diurnal rhythmicity analysis. To assess the effects of distinct circadian time frames on possible GH secretion differences between patients and controls, we divided the 24 h GH concentration series into six equal epochs in which we compared mean GH concentrations between patients and controls. The time epochs were defined as follows: I, 1630 h to 2030 h; II, 2030 h to 0030 h; III, 0030 h to 0430 h; IV, 0430 h to 0830 h; V, 0830 h to 1230 h; and VI, 1230 h to 1630 h.

Approximate entropy (ApEn). ApEn is a model-independent statistic used to quantify the regularity of a time series, in which is measured, within a predefined tolerance r given a pattern of window length m, the likelihood of a similar pattern in the next incremental window.[36] Greater regularity yields smaller ApEn values, whereas

greater independence among sequential values of a time series yields larger ApEn values. ApEn parameters of m = 1 and r = 20% of the intra-series standard deviation were used, the statistical suitability of which has been established previously.[36] Data are also presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same time series.

Statistical analysis. Results are expressed as mean ± standard error (SE) unless otherwise specified. Unpaired *t* tests and repeated measures analysis of variance (RM-ANOVA) were used to assess differences in means between the two groups. Pearson's correlation coefficient was applied to assess all correlations. The effects of group and time on total ghrelin levels were also tested using RM-ANOVA..All tests were two-tailed and significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS for Windows (release 16.0, SPSS, Inc., Chicago, IL).

RESULTS

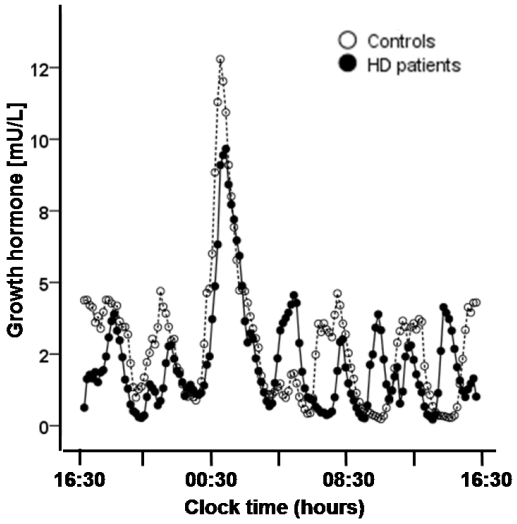
Subjects

The HD and the control group did not differ with respect to age, sex, BMI, body fat or lean body mass (all $p \geq 0.15$, **Table 1**). There were also no significant differences in serum HbA1c or urinary creatinine, epinephrine, norepinephrine and dopamine levels (all $p \geq 0.10$).

Deconvolution analysis of GH time series

Mean 24 h GH concentrations were not significantly different between HD patients and controls (2.20 ± 0.54 *vs.* 2.83 ± 0.59 mU/L, p = 0.441; **Figure 1**). This was also the case when each of the six circadian time frames was analyzed separately (all $p \geq 0.276$ by RM-ANOVA). The number of GH pulses as well as basal, pulsatile and total GH secretion rates tended to be lower in HD patients, but the differences did not reach statistical significance (all $p \geq 0.077$). Details of all deconvolution-derived GH secretory kinetics are presented in **Table 2**. Total 24 h GH production was not significantly associated with HbA1c levels in either patients or controls.

Figure 1. Mean serum GH concentrations in HD and control subjects. *Sampling started at 1630 h and was continued at 10-min intervals for 24 h.*



Regularity of serum GH concentration time series

The ApEn values of the serum GH time series were not significantly different between HD patients and controls (0.45 ± 0.07 *vs.* 0.52 ± 0.07, p = 0.488). The same held for ApEn ratios (0.39 ± 0.03 *vs.* 0.41 ± 0.03, p = 0.681). However, only in HD patients GH ApEn

Table 2. Deconvolution analysis of 24 h serum GH concentrations.

	HD patients†	Controls†	p-value‡
Half-life (min)	15.4 ± 1.1	15.5 ± 1.1	0.996
Pulse half-duration (min)	28.6 ± 4.0	27.2 ± 2.1	0.775
Pulse frequency (no./24 h)	14.6 ± 1.8	18.1 ± 0.5	0.077
Mean mass secreted per pulse (mU/L)	9.1 ± 1.9	9.7 ± 2.6	0.865
Basal production rate (mU/L/24 h)	$4.9 \times 10^{-3} \pm 9.3 \times 10^{-4}$	$7.7 \times 10^{-3} \pm 2.7 \times 10^{-3}$	0.354
Pulsatile production rate (mU/L/24 h)	130 ± 35	170 ± 40	0.459
Total production rate (mU/L/24 h)	137 ± 36	181 ± 43	0.439
Percent pulsatile (%)	93 ± 1.8	94 ± 0.9	0.623

†) Values are indicated as mean ± SE.
‡) Differences between groups were assessed by unpaired t-tests: * $p < 0.05$

values significantly correlated with GH mean levels (r = +0.78, p = 0.013), number of secretion bursts (r = +0.70, p = 0.039), total pulsatile secretion (r = +0.86, p = 0.003) and total secretion (r = +0.87, p = 0.002), while none of these relations was significant in controls (all $p \geq 0.227$). Results were similar when ApEn ratios were used instead of ApEn values (data not shown).

IGF-1 and IGFBP3 levels

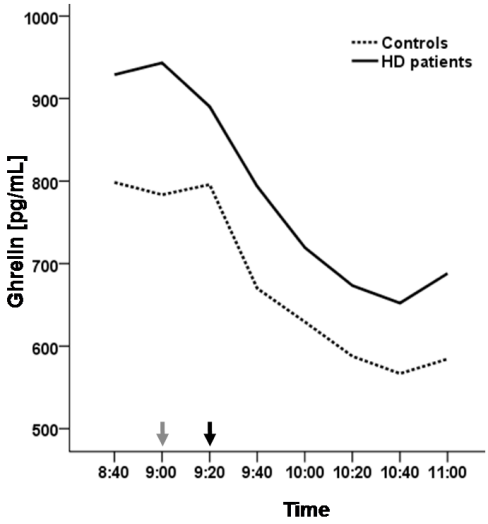
There were no significant differences between HD patients and controls in mean serum levels of either IGF-1 (19.01 ± 1.25 *vs.* 20.32 ± 2.75 nmol/L, p = 0.671) or IGFBP3 (3.97 ± 0.23 *vs.* 3.47 ± 0.21 nmol/L, p = 0.131). There was a trend for the association between IGF-1 and HbA1c levels in HD patients (r = +0.63, p = 0.068), but not in controls (r = +0.10, p = 0.791).

Ghrelin levels

Baseline ghrelin levels (defined as the mean concentration of ghrelin in three samples obtained immediately before breakfast) were not significantly different between HD and control subjects (922 ± 94 *vs.* 784 ± 86 pg/mL, p = 0.297), neither were mean ghrelin levels after meal consumption (731 ± 67 *vs.* 635 ± 51 pg/mL, p = 0.271). Ghrelin levels significantly decreased after the start of meal consumption (RM-ANOVA: $F(11) = 40.760$ and $p << 0.001$ for the time effect), but there was no group × time interaction effect ($F(11) = 0.718$ and p = 0.720) indicating similar rates of decrease in ghrelin levels between HD patients and controls (**Figure 2**). However, while mean ghrelin levels before and after breakfast did not correlate with GH secretion in either patients or controls, only in HD patients the ratio of post- to preprandial ghrelin levels was inversely related to GH mean levels (r

= -0.78, p = 0.014), total pulsatile secretion (r = -0.87, p = 0.002) and total secretion (r = -0.87, p = 0.002); i.e. postprandial ghrelin suppression was greater in HD patients with high GH secretion (**Figure 3**). On the other hand, the later relations were all *positive* and non-significant in controls (r between +0.33 and +0.49, all p ≥ 0.177) (**Figure 3**). Likewise, the ratio of post- to preprandial ghrelin levels was significantly associated with GH ApEn values in HD patients (r = -0.69, p = 0.038), but not in controls (r = -0.18, p = 0.647); i.e. greater postprandial ghrelin suppression was associated with more irregular GH secretion in HD. Ghrelin levels were not related to HbA1c levels in either patients or controls (all p ≥ 0.532).

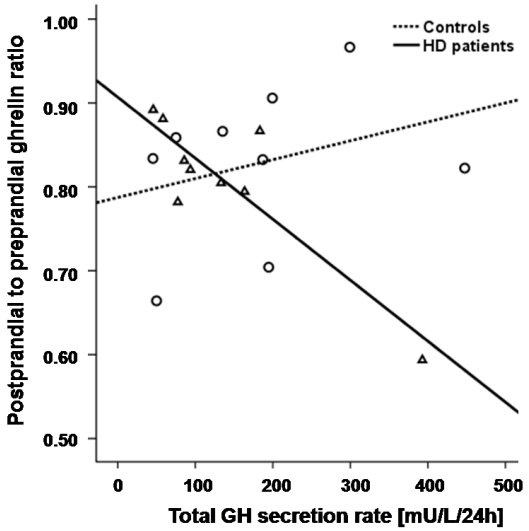
Figure 2. Mean plasma ghrelin concentrations before and after food intake in HD and control subjects. *Ghrelin levels were measured every 10 min from 0840 h to 1100 h (i.e. 20 min before to 2 h after the start of breakfast which was consumed between 0900 and 0920 h (gray and black arrow, respectively).*



GH secretion in relation to clinical phenotype

Greater daily GH secretion was significantly associated with a lower body weight in both HD patients and controls (**Table 3**). However, only in HD patients a higher GH secretion rate was also associated with a lower lean body mass. Moreover, in HD patients a higher total daily GH secretion rate was significantly related to a greater degree of motor as well as functional impairment (**Table 3**), while there was a trend for the association with CAG repeat size (r = +0.63, p =0.072). Similarly, higher GH ApEn values were associated with more severe scores on the UHDRS motor (r = +0.79, p = 0.012), total functional capacity (r = -0.89, p = 0.001), functional assessment (r = -0.84, p = 0.004), and independence (r = -0.92, p < 0.001) scales (**Figure 4**); results were similar when ApEn ratios were used instead of ApEn values (data not shown).

Figure 3. Postprandial ghrelin suppression and GH secretion. *Only in HD patients, postprandial ghrelin suppression was significantly associated with daily GH production (r = -0.87, p = 0.002).*



Ghrelin secretion in relation to clinical phenotype

Fasting ghrelin levels were not significantly associated with clinical phenotype, except for a significant inverse association with lean body

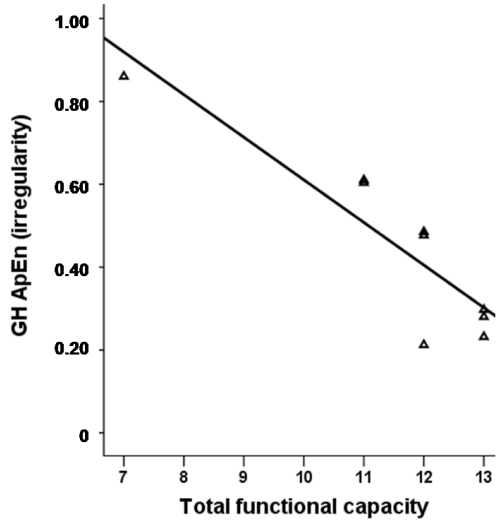
Table 3. Clinical correlates of GH and ghrelin secretion in Huntington's disease patients and controls

	GH (total 24 h secretion)		Ghrelin (post-/preprandial ratio)	
	HD patients†	Controls†	HD patients†	Controls†
Body weight [kg]	-0.83**	-0.84**	0.79*	0.02
BMI [kg/m²]	-0.55	-0.86**	0.59	-0.57
Fat mass [kg]	-0.36	-0.54	0.43	-0.78*
Lean body mass [kg]	-0.67*	-0.50	0.58	0.53
CAG repeat size	0.63	-	-0.69*	-
Motor score	0.75*	-	-0.68*	-
TFC	-0.92**	-	0.82**	-
FAS	-0.93**	-	0.87**	-
IS	-0.91**	-	0.78*	-

†) Values are indicated as Pearson's correlation coefficients: * $p < 0.05$, ** $p < 0.01$.
Abbreviations: BMI = Body Mass Index; FAS = Functional Assessment; GH = growth hormone; HD = Huntington's disease; IS = Independence Score; TFC = Total Functional Capacity.

mass in controls ($r = -0.68$, $p = 0.043$). However, a number of differences became apparent between HD patients and controls when the ratio between post- to preprandial ghrelin levels was assessed. In controls, this ratio was negatively associated with body fat mass, whereas in HD patients this ratio was positively, although not significantly, associated with body fat (**Table 3**). Conversely, in HD patients, but not in controls, the post- to preprandial ghrelin ratio significantly increased with higher body weight; i.e. postprandial ghrelin suppression was smaller in patients with a higher body weight. Moreover, in HD patients this ratio was also significantly related to the length of the CAG repeat mutation, as well as the degree of motor and functional impairment; i.e. postprandial ghrelin levels decreased relatively more in HD patients with a higher CAG repeat number, and a greater degree of motor and functional impairment (**Table 3**).

Figure 4. GH secretion regularity and functional capacity. *In HD patients, GH secretion becomes less regular (i.e. the approximate entropy increases) with decreasing total functional capacity ($r = -0.89$, $p = 0.001$).*



DISCUSSION

In this study we present the first detailed description of 24 h GH secretory dynamics in patients with HD. In addition, we provide a detailed description of ghrelin release in relation to food intake and GH secretion in these patients. Although mean GH and ghrelin secretion characteristics were not different between early stage HD patients and controls, there were interesting differences between the groups in the way GH and ghrelin secretion were related to body composition. Moreover, various GH and ghrelin secretion characteristics were related to disease severity in HD patients. Thus, our findings indicate subtle changes in the regulation of GH and ghrelin secretion dynamics in early stage HD patients that are likely to become more pronounced in the later stages of the disease.

The regulation of GH secretion is a complex process.[37] Most GH secretion occurs in pulses which are generated by interactions among GH-releasing hormone (GHRH), ghrelin and somatostatin under negative feedback control by both GH and IGF-1.[37] GHRH and somatostatin are secreted by specialized and interconnected mediobasal and periventricular neurons into hypophyseal portal blood and then transported to the anterior pituitary gland where GHRH stimulates and somatostatin inhibits GH release. Systemic GH and IGF-1 exert negative feedback on GH secretion primarily via hypothalamic actions, which enhance the secretion of somatostatin and limit the release of GHRH.[37] Ghrelin further amplifies GH release by acting on GH-secreting cells in the pituitary, as well as GHRH and somatostatin neurons in the hypothalamus.[37]

We found no major differences in GH secretion characteristics between our group of HD patients and controls. Therefore, it can be assumed that the system giving rise to pulsatile GH release is relatively intact in early stages of HD. However, there were strong associations between disease severity and GH secretion in the HD group. In addition, the regularity of GH secretion decreased (increasing ApEn) with worsening clinical phenotype in these patients, indicating that also the feedback control of GH release becomes less tight with continuing disease progress. A recent study in more advanced HD patients indeed found comparatively larger differences in both GH and IGF-1 levels than we found in our cohort of early stage patients.[38] These findings, therefore, suggest that the intricate interplay between GHRH and somatostatin neurons and their responsiveness to GH, IGF-1 and ghrelin is likely to become deranged with disease progression. Deregulated GH secretion is thus likely to underlie the exaggerated and paradoxical GH responses that have been reported previously in later stage HD patients.[12-23]

Ghrelin secretion is thought to be triggered by the sympathetic nervous system,[31] whereas as yet unidentified postgastric feedback mechanisms transmitting visceral information to the brain appear to underlie meal-related suppression of plasma ghrelin.[39] These ghrelin secretion pathways seem to be relatively spared in early stages of HD as fasting ghrelin levels as well as postprandial suppression of ghrelin release were not significantly different between early stage patients and controls. Importantly, however, while body fat mass was inversely associated with postprandial ghrelin suppression in controls, this relation was reversed in HD patients, accounting for the positive association between body weight and postprandial ghrelin suppression in this group. Moreover, postprandial ghrelin suppression also increased with a higher number of CAG repeats in the mutant *huntingtin* gene. These finding are interesting in view of our recent discovery of an increased rate of weight loss in both HD patients and R6/2 transgenic mice with higher CAG repeat number[3]; as ghrelin is a potent stimulator of food intake,[40] stronger inhibition of postprandial ghrelin release in HD patients with higher CAG repeat sizes may lead to less energy intake and, thereby, contribute to the increased rate of weight loss in these subjects.[3] Apart from CAG repeat size, more severe motor as well as functional impairment were also associated with a greater degree of postprandial ghrelin suppression, suggesting that despite the relative preservation of the mechanisms involved in ghrelin release in early stages of HD, ghrelin secretion may become deregulated in the later stages of the disease.

Ghrelin is the endogenous ligand of the GH secretagogue receptors and stimulates GH secretion when administered peripherally or centrally.[40] Nevertheless, ghrelin is thought not to be physiologically involved in the regulation of GH secretion, since its circulating levels are not correlated with those of GH.[41] Accordingly,

in our control group we found no significant associations between ghrelin and GH secretion characteristics. In the HD group, however, a greater degree of postprandial ghrelin suppression was associated with both higher, as well as more irregular GH secretion. These findings suggest that, in the context of HD, the GH secretory ensemble may become increasingly sensitive to peripheral signals such as ghrelin.

Progressive pathology of structures involved in the regulation of both GH and ghrelin secretion, particularly the GHRH and somatostatin neurons in the hypothalamus, may account for our findings.[2,11] Somatostatin immunoreactivity is indeed greatly reduced in the lateral tuberal nucleus of HD patients.[42] However, since the somatostatin neurons in the periventricular nucleus are thought to be primarily involved in the neurohumoral regulation of GH secretion,[43] further neuropathological studies are warranted to assess to what extent these neurons are affected in HD. Also a reduced number of hypocretin (also known as orexin) neurons has been found in the lateral hypothalamus of HD patients.[43-45] As plasma ghrelin can interact with hypocretin neurons[46] and hypocretin can inhibit GH secretion,[47] progressive loss of hypocretin neurons may also account for the association between deregulated GH and ghrelin secretion and disease severity in our cohort of HD patients.

A potential limitation of our study could be the assessment of hormone levels during one circadian cycle. However, considering that HD is a slowly progressive disorder and that homeostatic control mechanisms within the human somatotropic axis strongly preserve the day to day pattern of GH release across a wide spectrum of ages and body compositions,[48,49] our results are unlikely to have been affected by the assessment of only one circadian cycle. Another potential limitation of our study is the relatively small number of participants. Nevertheless, this limitation is offset by the rigorous assessment and modeling of diurnal hormone secretion patterns which is unfeasible in larger groups of subjects.

In conclusion, our findings suggest subtle changes in the regulation of GH and ghrelin secretion dynamics in early stage HD patients that may become more prominent in the later stages of the disease. The assessment of postprandial ghrelin suppression, in particular, is a relatively simple procedure that should be evaluated as a potential biomarker to assess disease progression in future studies.

ACKNOWLEDGMENTS

We are greatly indebted to: E. J. M. Ladan-Eygenraam, E. C. Sierat-van der Steen, M. van Dijk-Besling, and H. G. Haasnoot-van der Bent for technical assistance during the study, and P. Kok, C.C. de Wit and S. Vidarsdottir for their invaluable suggestions and comments. This work was supported by The Netherlands Organization for Scientific Research (grant number 017.003.098 to N.A.A.).

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