

# Huntington's Disease

*Hypothalamic, endocrine and metabolic aspects*

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*We are a wave, our calm is our naught,*

*We have no rest, lest we will be not.*

***Muhammad Iqbal*** (1877-1938 CE)







*To my father,*

*who taught me reason and perseverance*

*To my mother,*

*who bestowed me with love and inspiration*

*To Jorien,*

*for sharing the joys and sorrows of life with me*







# C ontents

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<b>Chapter 1</b>	<b>GENERAL INTRODUCTION &amp; AIMS OF THE THESIS</b>	<b>1</b>
	<i>Rev Neurosci (2007); 18: 223-251</i>	
<b>PART I</b>	<b>SECONDARY SIGNS OF HUNTINGTON'S DISEASE</b>	<b>43</b>
<b>Chapter 2</b>	Weight loss in Huntington's disease increases with higher CAG repeat number	45
	<i>Neurology (2008); 71(19): 1506-1513</i>	
<b>Chapter 3</b>	Normal and mutant <i>HTT</i> interact to affect clinical severity and progression in Huntington's disease	57
	<i>Neurology (2009); 73(16):1280-1285</i>	
<b>Chapter 4</b>	Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease	73
	<i>Parkinsonism Rel Disorders (in press)</i>	
<b>Chapter 5</b>	Autonomic symptoms in patients and premanifest mutation carriers of Huntington's disease	87
	<i>Eur J Neurol (in press)</i>	
<b>PART II</b>	<b>HYPOTHALAMIC PATHOLOGY IN HUNTINGTON'S DISEASE</b>	<b>99</b>
<b>Chapter 6</b>	Hypocretin and melanin-concentrating hormone in patients with Huntington's disease	101
	<i>Brain Pathol (2008); 18(4): 474-483</i>	
<b>PART III</b>	<b>ENDOCRINE STUDIES IN HUNTINGTON'S DISEASE</b>	<b>119</b>
<b>Chapter 7</b>	Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease	121
	<i>J Clinical Endocrinol Metab (2009); 94(4):1223-8</i>	
<b>Chapter 8</b>	Growth hormone and ghrelin secretion are associated with clinical severity in Huntington's disease	135

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	<i>Eur J Neurol (in press)</i>	
<b>Chapter 9</b>	Altered thyrotropic and lactotropic axes regulation in Huntington's disease <i>Clinical Endocrinol (Oxf.) (in revision)</i>	149
<b>Chapter 10</b>	Leptin secretion rate increases with higher CAG repeat number in Huntington's disease patients <i>Clinical Endocrinol (Oxf.) (in press)</i>	161
<b>Chapter 11</b>	Delayed onset of the diurnal melatonin rise in patients with Huntington's disease <i>J Neurol (2009) 256:1961–1965</i>	173
<b>PART IV</b>	<b>METABOLIC STUDIES IN HUNTINGTON'S DISEASE</b>	<b>183</b>
<b>Chapter 12</b>	Systemic energy homeostasis in Huntington's disease patients <i>J Neurol Neurosurg Psychiatry (in press)</i>	185
<b>Chapter 13</b>	<b>SYNOPSIS, CONCLUSIONS &amp; FUTURE PERSPECTIVES</b>	<b>197</b>
<b>APPENDICES</b>		<b>205</b>
<b>A</b>	Summary and conclusions in Dutch	207
<b>B</b>	Excerpt in Persian	215
<b>C</b>	Acknowledgments	217
<b>D</b>	Curriculum vitae	219
<b>E</b>	List of publications	221

# GENERAL INTRODUCTION & AIMS OF THE THESIS

*Partly based on:*

**Aziz NA, Swaab DF, Pijl H, Roos RA. (2007).** Hypothalamic dysfunction and neuroendocrine and metabolic alterations in Huntington's disease: clinical consequences and therapeutic implications. *Rev Neurosci.* 18(3-4): 223-251.



*“...The hereditary chorea, as I shall call it, is confined to certain and fortunately a few families, and has been transmitted to them, an heirloom from generations away back in the dim past. It is spoken of by those in whose veins the seeds of the disease are known to exist, with a kind of horror; and not at all alluded to except through dire necessity, when it is mentioned as ‘that disorder’. It is attended generally by all the symptoms of common chorea, only in aggravated degree hardly ever manifesting itself until adult or middle life, and then coming on gradually but surely, increasing by degrees, and often occupying years in its development, until the hapless sufferer is but a quivering wreck of his former self... I know nothing of its pathology. I have drawn your attention to this form of chorea gentlemen, not that I consider it of any great practical importance to you, but merely as a medical curiosity, and as such it may have some interest.”*

**George Huntington** *On chorea. Medical and Surgical Reporter. 1872; Vol. XXVI., pp. 317-21.*



## **1. Introduction**

## **2. Characteristics of weight loss, sleep disturbances and autonomic dysfunction in HD**

*2.1. Weight loss*

*2.2. Sleep disturbances*

*2.3. Autonomic nervous system dysfunction*

## **3. Hypothalamic dysfunction in HD**

*3.1. Hypothalamic pathology in HD*

*3.2. Aminergic and neuropeptidergic alterations in HD brains suggestive of hypothalamic dysfunction*

*3.2.1. Histamine*

*3.2.2. Somatostatin and neuropeptide Y*

*3.2.3. Neurotensin and thyrotropin-releasing hormone*

*3.2.4. Gonadorelin*

*3.2.5. Hypocretin (orexin)*

*3.2.6. Corticotropin-releasing hormone*

*3.2.7.  $\alpha$ -Melanocyte-stimulating hormone*

## **4. Endocrine abnormalities in HD**

*4.1. Hypothalamo-neurohypophysial system*

*4.2. Hypothalamo-pituitary-adrenal (HPA) axis*

*4.3. Somatotrophic axis*

*4.4. Lactotropic axis*

*4.5. Hypothalamo-pituitary-thyroid (HPT) axis*

*4.6. Hypothalamo-pituitary-gonadal (HPG) axis*

*4.7. Ghrelin and leptin*

## **5. Systemic metabolic abnormalities in HD**

*5.1. Carbohydrate metabolism*

*5.2. Lipid metabolism*

*5.3. Protein metabolism*

*5.4. Basal metabolism and energy expenditure*

## **6. Conclusions**

## **7. Aims of the thesis**

## 1. INTRODUCTION

Huntington's disease (HD) is an autosomal dominant progressive, neurodegenerative disorder caused by an expanded trinucleotide (CAG) repeat in the HD (*HTT*) gene, located on the short arm of chromosome 4. Unwanted choreiform movements, psychiatric and behavioural problems and cognitive impairment characterize the disease.<sup>74</sup> Other less well-known, but debilitating manifestations of HD include weight loss, sleep disturbances and autonomic nervous system (ANS) dysfunction. Only little is known about the exact pathophysiological mechanisms underlying these symptoms. In recent years, novel insights have emerged from both animal and human studies indicating the existence of substantial hypothalamic pathology and dysfunction in HD, even in an early phase of the disease.<sup>94</sup> Considering the pivotal role of the hypothalamus in the regulation of systemic energy homeostasis, sleep-wake states and ANS functions,<sup>92,207,208,232,236</sup> hypothalamic dysfunction per se as well as secondary (neuro)endocrine and metabolic alterations could partly explain the pathogenesis of emaciation, sleep disturbances and autonomic failure in patients with HD; nonetheless, the anatomical and functional abnormalities of the hypothalamus in HD have not been systematically studied in conjunction with these and other clinical parameters. Therefore, after summarizing the precise characteristics of weight loss, sleep and ANS problems in HD, this review aims to: 1) describe the precise nature of hypothalamic dysfunction and neuroendocrine and metabolic alterations in HD, and 2) link these abnormalities to the aforementioned clinical manifestations, thereby providing rationale for future animal and clinical studies on the relation between hypothalamic dysfunction and symptomatology in HD. Assuming that hypothalamic dysfunction and consequent neuroendocrine and metabolic abnormalities could contribute to the weight loss and disturbances of sleep and autonomic functions in HD, some therapeutic strategies will be discussed as well.

## 2. CHARACTERISTICS OF WEIGHT LOSS, SLEEP DISTURBANCES AND AUTONOMIC DYSFUNCTION IN HD

### 2.1. Weight loss

Weight loss frequently complicates HD and is particularly marked in the later stages of the disease.<sup>112,140</sup> Several studies, including clinical follow-up<sup>187</sup> and cross-sectional prevalence studies<sup>52,181,215</sup>, anthropometric measurements with dietary evaluations<sup>62,63,216</sup> as well as one post-mortem study of 217 cases,<sup>149</sup> have all shown that many HD patients are either underweight or tend to lose weight in the course of their illness and eventually become cachectic. An exception is the recent study by Hamilton et al.<sup>73</sup> that assessed weight changes in 927 adults with HD who were followed prospectively for about 3 years. The authors found that most HD patients maintained their weight and that, on the whole, the patient group gained approximately 0.11 kg per year. However, on average their entire group of HD patients gained less weight than the United States national average, as per the third National Health and Nutrition Examination Survey,<sup>73</sup> and weighed almost 10 kg less than a community sample of comparably aged adults.<sup>73</sup> It is very likely that clinical information not available for that study—for example, the introduction of nutritional supplements, institutional placement, and types of drugs used (notably neuroleptics and antidepressants)—could account for the surprising pattern of weight change found by Hamilton et al.

In contrast to the weight loss associated with most other diseases, the emaciation in HD is not accompanied by anorexia but usually by an increased appetite.<sup>62,63,146,187,216</sup> An increased caloric intake could possibly also underlie the peculiar observation of an inverse relation between the age-at-onset of the disease and the daily milk consumption in Dutch HD patients<sup>32</sup> since the onset of HD could be marked by an increased demand for calories. Furthermore, in a longitudinal, prospective study of 30 patients with HD, Trejo et al.<sup>215</sup> found that although nutritional supplementation (i.e. 473 kcal added to the regular diet) over a 90-day-period could stabilize or slightly improve body weight, it could not induce a significant change in body mass index in 87% of the patients. These findings support the notion that HD patients have an increased energy requirement.<sup>146</sup>

Little is known about the pathophysiological mechanisms underlying weight loss in HD. Although dysphagia can complicate food intake,<sup>91,124,216</sup> and there are indications of a higher sedentary energy expenditure due to increased spontaneous physical activity (unwanted movements),<sup>68,172,203</sup> these findings do not explain the lower body mass index (BMI) found in certain groups of asymptomatic subjects at-risk for HD<sup>62,63</sup> nor do they account for the lower body weight of those HD patients who are at an early stage of the disease when both dysphagia and unwanted movements are still absent.<sup>52</sup> Moreover, it appears that HD patients tend to lose most weight in the final *hypokinetic* stages of the disease,<sup>187</sup> although weight gain may occur in some advanced-stage patients.<sup>146</sup> Weight loss frequently leads to progressive general weakening, resulting in an increasingly higher risk of developing co-morbidity, which causes a further decline in the patient's quality of life.<sup>146</sup> A survey of patients in Venezuela demonstrated that a substantial number of them were malnourished,<sup>140</sup> and in the United States nutritional deficiencies cause or contribute to death more frequently in HD patients than in controls.<sup>118,119</sup> Moreover, one study has demonstrated that a higher body weight in the early stages of the disease is associated with slower disease progression.<sup>145</sup> Therefore, the mechanisms which cause weight loss in HD may also underlie and/or aggravate the neurodegenerative process per se. In this regard dysfunction and pathology of various hypothalamic structures, notably the nucleus tuberalis lateralis (NTL) and the lateral hypothalamus, are likely to be involved (refer to the section 'Hypothalamic pathology in HD').

## 2.2. Sleep disturbances

From a postal self- or carer-completion questionnaire community survey Taylor et al.<sup>212</sup> estimated the prevalence of sleep problems to be almost 90 percent in HD patients. Nearly two-thirds of the respondents rated the sleep problems as either 'very' or 'moderately' important contributing factors to overall problems. Moreover, the majority of the principal carers also reported significant sleep disruption, primarily attributable to the patients' sleep problems, which adversely affected their relationship with the patient.<sup>212</sup> Polysomnography in patients with HD showed sleep fragmentation in moderately to severely affected cases, with progressive deterioration as the disease progresses.<sup>231</sup> Sleep efficiency (i.e. the ratio between time spent asleep and total amount of time spent in bed) has been reported as varying from 48% to 80%, with frequent nocturnal awakenings, an increase of stage I non-rapid eye movement (NREM) sleep and a reduced amount of slow wave sleep.<sup>39,164,199,231</sup> Also a shortening of rapid eye movement (REM) sleep latency, which is a cardinal characteristic of the sleeping pattern in narcolepsy patients, has been documented<sup>86</sup> as well as a significant increase in sleep spindle density during phase II NREM sleep.<sup>58,199</sup> Two recent studies applying wrist actigraphy demonstrated dysregulation of the



sleep-wake cycle and circadian timing in HD patients.<sup>84,141</sup> During sleep, patients not only showed significantly more activity and spent more time making high acceleration movements, but they also made significantly more movements than control subjects.<sup>84</sup> Although the presence of dyskinesia<sup>66</sup>, medication effects and depression may contribute to sleep disturbances observed in HD,<sup>39</sup> the precise pathogenetic mechanisms responsible for sleep disturbances in HD patients remain largely unexplored. Particularly disease-specific neurodegenerative changes affecting (hypothalamic) structures regulating sleep-wakefulness have not been systematically investigated. However, changes in the suprachiasmatic nucleus (SCN) and (functional) alterations in the hypothalamic histaminergic and orexinergic systems in HD are probably among the mechanisms involved (for further details refer to the sections ‘Hypothalamic pathology in HD’ and ‘Aminergic and neuropeptidergic alterations in HD brains suggestive of hypothalamic dysfunction’). Given the high prevalence of sleep disorders in HD and their devastating impact on the quality of life of both patients and carers,<sup>212</sup> it is of the utmost importance to elucidate the underlying pathogenic pathways in order to devise effective therapeutic strategies.

### 2.3. Autonomic nervous system dysfunction

Vegetative symptoms indicative of autonomic nervous system (ANS) dysfunction have repeatedly been reported in patients with HD and include defects in postural vasoregulatory mechanisms,<sup>2,3</sup> hyperhydrosis of hands and feet, and disturbances of micturition,<sup>28</sup> and swallowing difficulties.<sup>91,124</sup> Although vegetative symptoms are most prominent in the advanced stages of the disease,<sup>99,146</sup> autonomic complaints such as dizziness following standing up, excessive perspiration and tachycardia can occur even in mildly disabled HD patients (i.e. Shoulson and Fahn stages I and II<sup>196</sup>) and even in otherwise asymptomatic gene carriers.<sup>100</sup>

Several studies have investigated ANS function in HD patients by means of a series of standardized tests, such as the blood pressure response to sustained handgrip,<sup>50,100</sup> the pupillary light reflex latency,<sup>50</sup> orthostatic blood pressure test,<sup>6,50,100,192</sup> heart rate variability at rest and during the Valsalva maneuver, and the sympathetic skin response.<sup>6,50,192</sup> Compared to controls, the blood pressure response to sustained handgrip, a test of sympathetic function, was diminished in the patient group with a relatively long duration of disease.<sup>50,100</sup> In contrast, the blood pressure response to sustained handgrip in a group of *mildly* disabled HD patients was elevated.<sup>100</sup> The pupillary light reflex latency<sup>50</sup> as well as the sympathetic skin response (SSR) latencies<sup>6,192</sup> were prolonged in HD patients, suggesting parasympathetic and sympathetic dysfunction, respectively. The SSR amplitudes were also diminished in the patient group.<sup>6,192</sup> Overall, patients showed lower heart rate variability (HRV) indices than controls.<sup>6,192</sup> Spectral analysis of HRV, which yields several frequency bands of interest, demonstrated sympathetic *hyper*activity in the *asymptomatic* gene carriers by a higher power of low frequency band (LFB; 0.04-0.15 Hz).<sup>100</sup> Similarly, a higher power of LFB and an increase in the ratio between LFB to high frequency band (HFB; 0.15-0.40 Hz) was demonstrated in *mildly disabled* HD patients.<sup>100</sup> LFB in the supine position reflects both the sympathetic and parasympathetic activities and HFB reflects mainly vagal activity. A higher LFB/HFB ratio, which can be considered as a marker of sympathovagal balance, indicates a relatively higher cardiac sympathetic activity in the group of *mildly* affected HD patients.<sup>100</sup> However, ANS *hypo*function, predominantly parasympathetic dysfunction, was found in more advanced HD patients,<sup>100</sup> although an earlier study of parasympathetic function in HD had not found any abnormalities.<sup>34</sup> Moreover, the prolonged SSR latencies, smaller amplitudes, and lower HRV detected in HD patients by some investigators seemed to closely

correlate with various components of the Unified Huntington Disease Rating Scale (UHDRS) scores.<sup>6,192</sup> The exact origin of impaired ANS function in individuals with HD remains to be elucidated.

### 3. HYPOTHALAMIC DYSFUNCTION IN HD

Emaciation, sleep disturbances and autonomic dysfunction are on the one hand important indicators of the rate of disease progression and on the other hand these symptoms can considerably diminish the quality of a patient's life. Therefore, it is important to elucidate the pathophysiology of these symptoms in order to strike novel and more effective therapeutic targets. As mentioned before, the precise aetiology of these symptoms in HD is largely unknown. Nevertheless, several lines of evidence derived from studies in both patients with HD and HD animal models indicate that HD is accompanied by considerable hypothalamic dysfunction, even in the very early stages of the disease. Since the hypothalamus is the main control centre in the brain involved in the regulation of body energy homeostasis, sleep-wake cycles and the coordination of autonomic functions,<sup>89,92,207,208,232,236</sup> we propose that hypothalamic dysfunction per se as well as subsequent (neuro)endocrine and metabolic abnormalities in patients with HD may be the primary underlying cause of the aforementioned symptoms or substantially contribute to their pathogenesis. In order to substantiate this hypothesis, we will give an overview of all the findings suggestive of hypothalamic dysfunction in HD and attempt to link them to the HD symptomatology with a special focus on weight loss, sleep and autonomic disturbances.

#### 3.1. Hypothalamic pathology in HD

A comprehensive summary of hypothalamic changes in HD patients, found in post-mortem brain preparations, has been given by Swaab.<sup>208</sup> In short, qualitative changes are presumed in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN),<sup>188,227</sup> the ventromedial nucleus (VMN) and the lateral hypothalamus.<sup>29</sup> Normally, the neurons of the PVN project not only to the neurohypophysis and the median eminence, but also to various brainstem nuclei as well as to the intermediolateral columns of the spinal cord, and in this way modulate and coordinate various autonomic functions,<sup>92</sup> including autonomic innervation and control of adipose tissue.<sup>67,109,110</sup> Moreover, the PVN, VMN and the lateral hypothalamus are tightly involved in the physiological regulation of body energy homeostasis. So, pathology of these structures in HD could cause symptoms such as weight loss and autonomic failure. However, the cited papers about hypothalamic changes in HD generally describe a single<sup>188</sup> or a few cases,<sup>29,227</sup> while systematic morphological studies, such as performed for the NTL (see further), have not been reported for the PVN, VMN or the lateral hypothalamus (H.P. Kremer, thesis). Quantitatively, no significant neuronal loss was found in the nucleus basalis of Meynert.<sup>41</sup> Kremer et al.<sup>111,113,114</sup> elaborated on the previous reports of a striking cell loss in the NTL of HD patients<sup>228,229</sup> and found that the NTL is indeed consistently affected in this disorder: up to 90% of the neurons in the NTL were lost in HD brains, while the remaining neurons showed features of degeneration.<sup>113,114</sup> Moreover, they found astrogliosis with an unchanged number of astrocytes, whereas the number of oligodendrocytes was reduced by 40%. The neurons of the tubero-mammillary nucleus (TMN) seemed to be largely well preserved.<sup>113</sup> The log-transformed neuronal counts in the NTL of HD patients correlated closely with age at death ( $n = 16$ ;  $r = 0.66$ ;  $p < 0.01$ ) and

age of onset ( $n = 16$ ;  $r = 0.78$ ;  $p < 0.001$ ), but neither with the duration of the disease, nor with the severity of the neostriatal changes.<sup>114</sup> Patients who had died young or who first displayed motor disturbances at an early age had considerably fewer neurons left than HD patients who had died in old age.<sup>114</sup> The authors hypothesized that the NTL is entwined in a network of structures involved in the regulation of feeding and weight and that weight loss in the course of HD is the expression of NTL pathology<sup>112</sup> since the loss of NTL neurons is less marked in patients with an older age at onset of the disease and the fact that late-onset patients tend to have a higher BMI at initial examination which is associated with a slower rate of disease progression.<sup>145</sup> Recently, the neuropathological data obtained from the aforementioned studies were extended by an in vivo study which demonstrated, by using magnetic resonance imaging and the technique of voxel based morphometry, that even in the early stages of the disease considerable hypothalamic neuronal atrophy is present in HD patients,<sup>94</sup> which is in line with the view that hypothalamic changes are both consistent and early features of HD.

In HD mouse models considerable anatomical and/or functional hypothalamic alterations have also been identified recently [107,127,141,156,162]. For example, Morton et al.<sup>141</sup> found a profound disintegration of circadian behaviour in the R6/2 mouse strain which was accompanied by marked disruption of expression of the circadian clock genes *mPer2* and *mBmal1* in the suprachiasmatic nuclei and associated with reduced SCN expression of prokineticin 2, a transcriptional target of *mBmal1* encoding a neuropeptide that normally suppresses daytime activity in nocturnal mammals<sup>141</sup>. These animal data are thus also in favour of the notion that pronounced hypothalamic changes may be a consistent feature of HD.

Exactly why the hypothalamus is involved in HD is not known. However, one intriguing possibility could be the abnormal interaction of mutant huntingtin with huntingtin-associated protein 1 (Hap1) which is more abundantly expressed in the hypothalamus than in other brain regions.<sup>194</sup> Inhibition of Hap1 expression in vitro decreases epidermal growth factor receptor signalling and cell viability whereas overexpression of Hap1 enhances this signalling activity and inhibits mutant huntingtin-mediated cytotoxicity.<sup>127</sup> Moreover, deletion of *Hap1* causes the selective degeneration of hypothalamic neurons.<sup>127</sup> Interestingly, recently it was demonstrated that Hap1 is involved in intracellular trafficking of the GABA<sub>A</sub> receptor, and that insulin exerts its feeding-inhibitory actions by downregulating Hap1 expression. Decreasing the expression of mouse hypothalamic Hap1 caused a decrease in food intake and body weight.<sup>194</sup> A link between mutant huntingtin expression, hypothalamic degeneration and weight loss may therefore be provided by Hap1.<sup>161</sup>

It is noteworthy that as yet intraneuronal aggregates of mutant huntingtin, the neuropathological hallmark of the disease, have not been studied systematically in the hypothalamus of patients with HD. Systematic assessments of polyglutamine aggregates in HD hypothalami could provide valuable insights into which hypothalamic loci are predominantly involved in HD, thereby providing precedent for more targeted evaluations of specific hypothalamic nuclei. Although it should be emphasized that the exact role of polyglutamine aggregates in cell death and dysfunction in HD is still much debated.<sup>9,22,201</sup> In the following section a recapitulation will be given of the aminergic and neuropeptidergic (post-mortem) findings in the brains of HD patients which appear to be of relevance for understanding the pathophysiology of, among other things, weight loss, sleep problems and vegetative symptoms in HD.

### 3.2. Aminergic and neuropeptidergic alterations in HD brains suggestive of hypothalamic dysfunction

#### 3.2.1. Histamine

The distribution of histamine H2- and H3-receptors have been assessed in both control cases and patients with HD by means of autoradiographic techniques.<sup>72,133</sup> In healthy subjects, the highest levels of histamine H2-binding sites were found in the caudate, putamen, and accumbens nuclei,<sup>133</sup> and the highest levels of histamine H3-binding sites were detected within the external and internal segments of the globus pallidus, substantia nigra and the striatum (within the striatum, H3-binding was noticeably higher in both the nucleus accumbens and the acetylcholinesterase-deficient striosomes).<sup>72</sup> In HD patients the levels of histamine H2-receptor binding sites were found to be markedly decreased in virtually all brain regions investigated, particularly in the putamen and globus pallidus lateralis. Furthermore, the loss of H2-binding sites was found to be related to the grade of the disease.<sup>133</sup> Comparably, values for H3-binding were also significantly lower in the caudate nucleus, putamen and both the external and internal globus pallidus, although not the insular cortex, in HD cases.<sup>72</sup> Since the TMN, the only site in the brain where histamine-releasing neurons are located,<sup>25</sup> does not show a clear cell loss in this disorder,<sup>113</sup> a functional change of the histaminergic system may be expected in HD.<sup>208</sup> As the histaminergic system plays a major role in the maintenance of wakefulness,<sup>25,198</sup> its dysfunction could underlie some features of sleep complaints in HD patients such as, and in particular, daytime sleepiness.<sup>212</sup> Histaminergic neural circuits are also involved in the control of energy balance.<sup>134,214</sup> However, activation of H2 receptors inhibits food intake, and H3 receptor knock out mice are obese and insulin resistant,<sup>130,214</sup> suggesting that the diminution of these receptors in HD is a compensatory consequence rather than a cause of weight loss. It should be noted though that perturbations have also been described in other, extra-hypothalamic, aminergic systems which too are involved in the coordination of sleep-wake cycles and food intake,<sup>198</sup> such as the noradrenergic<sup>14,38,235</sup> and the serotonergic pathways<sup>135</sup>, which could likewise contribute to the pathogenesis of sleep disruptions and weight loss in HD subjects.

#### 3.2.2. Somatostatin and neuropeptide Y

Somatostatin (SST) 1-12 immunoreactivity is normally abundantly present in the neurites and perikarya of the NTL, while SST immunoreactivity is greatly reduced in the NTL of HD patients.<sup>213</sup> In general, higher staining intensity is present in HD patients who have more NTL neurons left than in those who have fewer NTL neurons left. The data obtained so far suggest that NTL neurons cease to express SST-like peptides quite some time before their actual disappearance.<sup>208,213</sup> Since the SST neurons in the periventricular nucleus are thought to be primarily involved in the neurohumoral regulation of growth hormone (GH) secretion,<sup>71</sup> it is unclear to what extent elevated plasma GH levels observed in HD patients (see 'Endocrine abnormalities in HD') can be attributed to diminished SST availability in the NTL of HD subjects. In contrast to the diminished levels of NTL SST, the concentrations of both SST and neuropeptide Y (NPY) are markedly increased in the basal ganglia of patients with HD<sup>7,11-13,48,136</sup> which is consistent with selective preservation of a population of aspiny neurons in which both SST and NPY are colocalized<sup>48</sup>. Beal et al.<sup>13</sup> examined both SST-like immunoreactivity

and NPY-like immunoreactivity in 24 pathologically graded HD cases and 12 controls. Both SST and NPY immunoreactivity were significantly increased (about threefold) in the caudate, putamen, and the nucleus accumbens. Increases of SST and NPY immunoreactivity in mild and severe grades were similar indicating a relative but not absolute sparing of striatal aspiny neurons in which SST and NPY are colocalized.<sup>13</sup> Moreover, because neuropeptide changes were seen in the nucleus accumbens in early grade cases, when there is relatively little tissue atrophy, it is probable that not atrophy alone but also some other process, such as increased terminal sprouting, could contribute to the increased levels seen in these cases.<sup>13</sup> Among the other subcortical regions examined, SST immunoreactivity was significantly increased in the external pallidum, red nucleus, and locus ceruleus, whereas significant increases of NPY immunoreactivity were found in the external pallidum, subthalamic nucleus, substantia nigra compacta, claustrum, anterior and dorsomedial thalamus, bed nucleus of stria terminalis and locus ceruleus.<sup>13</sup> Interestingly, the locus ceruleus is known to contain NPY neurons, indicating that this nucleus could provide increased NPY innervation to other regions.<sup>13</sup> Except for the subthalamic nucleus, the NPY concentrations have not yet been determined in other structures of the hypothalamus of HD patients. NPY is a naturally occurring appetite transducer, and under normal conditions NPY-ergic transmissions represent an essential component of the common final pathway in the hypothalamic integration of energy homeostasis.<sup>92</sup> Although NPY-producing neurons are normally located in several sites in the brain, two subpopulations, one representing the extra-hypothalamic cluster in the brainstem including the locus ceruleus, and the other located in the hypothalamus along the length of the infundibular (or arcuate) nucleus and in the dorsomedial nucleus, apparently participate in a disparate manner in the daily management of food intake.<sup>92</sup> Regarding the disturbed systemic energy balance in HD, it would be very interesting to know whether perturbations also exist in the *hypothalamic* NPY-ergic system of these patients, especially the NPY neurons located in the infundibular nucleus, since animal studies suggest that huntingtin, the protein product of the *HTT* gene, may fulfil a physiological role in the homologue of this structure (i.e. the arcuate nucleus) in rodents.<sup>77</sup>

### 3.2.3. *Neurotensin and thyrotropin-releasing hormone*

Nemeroff et al.<sup>147</sup> measured the concentrations of neurotensin (NT) and thyrotropin-releasing hormone (TRH) by means of radioimmunoassay in post-mortem brain samples from patients with HD. Compared to patients without psychiatric or neurological disease, the patients with HD showed significantly elevated concentrations of these two neuropeptides in the nucleus caudatus, while in the amygdala only TRH levels were elevated.<sup>147</sup> However, it is unclear to what extent the elevated NT and TRH levels might reflect increased hypothalamic production of these neuropeptides. Apart from its many other physiological functions, hypothalamic NT seems to be implicated in the regulation of energy homeostasis, particularly in the anorexigenic networks of the hypothalamus.<sup>92</sup> Moreover, recently Cui et al.<sup>44</sup> demonstrated that all the anorexigenic hormones leptin, insulin, and  $\alpha$ -melanocyte-stimulating hormone directly induce NT gene expression in immortalized hypothalamic cell lines, which is in support of the notion that NT may have a direct role in the neuroendocrine control of feeding and energy homeostasis.<sup>44</sup> Increased hypothalamic NT levels may thus underlie the disturbed energy regulation in HD subjects as well as some endocrine (particularly hypercortisolism) and metabolic derangements in these patients (for further details refer to the sections ‘Endocrine abnormalities in HD’ and ‘Metabolic abnormalities in HD’) as NT dose-dependently increases adrenocorticotrophic hormone (ACTH) secretion and causes an increase in blood corticosterone and glucose concentration.<sup>217</sup> In addition, NT exerts other potent central nervous



system effects including hypothermia, antinociception, and modulation of dopamine neurotransmission. Peripherally, it acts as a paracrine and endocrine peptide of both the digestive and cardiovascular systems.<sup>217</sup> TRH is a tripeptide hormone which induces the secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary and, at least in humans, can also act as a prolactin-releasing factor.<sup>148</sup> Thus elevated levels of TRH in the caudate nucleus of HD patients could possibly be secondary to increased hypothalamic production of this peptide, which in turn may partly explain the deviant response to the TRH-stimulation test and the elevated basal plasma prolactin levels in HD subjects found by some investigators.<sup>75</sup> In addition, TRH is directly involved in the complex hypothalamic networks that establish energy balance by modulation of food intake, satiety, thermogenesis, and other autonomic responses.<sup>1,93</sup> Elevated hypothalamic levels of both NT and TRH could therefore partly account for the negative energy balance observed in HD<sup>52,172</sup> as well as some of the autonomic dysfunctions, especially disturbed vasomotion and thermoregulation, which can give rise to symptoms such as orthostatic hypotension and dyshydrosis in some of these patients.<sup>3,6,28</sup>

### 3.2.4. *Gonadorelin*

Gonadorelin or Gonadotropin-Releasing Hormone (GnRH) is a decapeptide hormone released by the hypothalamus that stimulates the synthesis and secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the adenohypophysis. GnRH concentrations have been reported to be increased fourfold in the median eminence of *female* choreic patients as compared with controls.<sup>18</sup> However, there was no difference in the median eminence levels of GnRH of the *male* choreic patients when compared with controls. Although the authors and others,<sup>178</sup> including George Huntington himself in his seminal paper, present some anecdotal descriptions of increased libido among HD patients,<sup>18,83</sup> a retarded menarche<sup>151</sup> but increased fertility among the female choreic as compared to her nonchoreic sibling,<sup>178</sup> later studies mainly suggest a high incidence of hypoactive sexual disorder in HD, notwithstanding the fact that certain HD patients may exhibit increased sexual interest and paraphilias.<sup>64</sup> It is questionable, though, whether the behavioural alterations have anything to do with changes in GnRH levels in HD, as they could also be accounted for by other mechanisms such as pathology of the prefrontal cortex in this disorder.<sup>191</sup>

Lavin et al.<sup>120</sup> performed a GnRH stimulation test in 8 HD patients and 10 controls, but found no clear differences or obvious abnormalities indicating intact pituitary responsiveness and gonadotropin release, although because of small subgroups (i.e. males, premenopausal and postmenopausal females) the data from the GnRH test were difficult to analyze. The findings of Bird et al.<sup>18</sup> seem to be at odds with those of others who found normal 24-hour curves of LH-secretion<sup>54</sup> and those of Markianos et al.<sup>132</sup> who found normal plasma levels of FSH but decreased plasma levels of both LH and testosterone in male HD patients. However, sex differences cannot be ruled out as the findings of Bird et al.<sup>18</sup> pertained only to the female patients. Moreover, the testosterone plasma concentrations correlated negatively with the severity of the disease and low testosterone levels were associated with dementia but not with depression or psychotic features.<sup>132</sup> The expected positive correlations of LH and FSH to age and the negative correlation of testosterone to age were, however, present in the patient samples studies by Markianos et al.<sup>132</sup> and the authors proposed that, in addition to blunted LH secretion, the direct neural hypothalamic–testicular pathway that interferes with synthesis and secretion of testosterone in the Leydig cells, independent of the pituitary, could be involved, especially for explaining the great reductions in

testosterone in patients with severe symptomatology in whom LH levels were found to be normal. A possible explanation for the increased immunoreactive GnRH concentrations in HD hypothalami<sup>18</sup> could thus be a decreased release of this neuropeptide from the hypothalamus. Nonetheless, recently a progressive reduction in the numbers of GnRH-immunoreactive neurons in the medial septum, diagonal band of Broca and medial preoptic area of the hypothalamus of the R6/2 mouse model of HD has been found starting at 5 weeks of age, prior to the onset of overt motor symptoms at 7-8 weeks of age, and becoming statistically significant with only 10% of the neurons remaining by 9 weeks of age.<sup>156</sup> Therefore, both the neuropathological data and the clinical findings concerning the hypothalamo-gonadal axis function in HD patients need further clarification.

### 3.2.5. *Hypocretin (orexin)*

Recently it was demonstrated that there is a significant loss and atrophy of hypocretin-immunostained neurons in the lateral hypothalamus of post-mortem HD brain preparations,<sup>162</sup> extending an earlier report of qualitative changes in this hypothalamic structure.<sup>29</sup> Hypocretin, a novel neuropeptide discovered in 1998, can be synthesized in the brain exclusively by neurons in the lateral hypothalamus.<sup>186</sup> This neuropeptide appears to play a crucial role in the regulation of sleep-wake cycles, body energy metabolism and autonomic functions,<sup>92,232</sup> partly by modification of the endocrine system including influences on the corticotropic, somatotropic and thyrotropic axes.<sup>103,104,155</sup> Hypocretin loss might therefore partly account for sleep, ANS and body weight abnormalities in patients with HD. However, four recent papers reported normal hypocretin-1 concentrations in the cerebrospinal fluid (CSF) of HD patients.<sup>10,21,69,137</sup> The apparent discrepancy between these latter findings and those of Petersen et al.<sup>162</sup>, who found a 27 % loss of hypocretin neurons in HD brains, could be accounted for by the fact that in human brain tissue the reduction in hypocretin-positive cell number was not as striking as in the concurrently studied R6/2 mouse model (27% vs. 72%).<sup>162</sup> This comparatively modest reduction in cell number appears not to be reflected in the CSF of HD patients, possibly due to compensatory mechanisms whereby the remaining hypocretin neurons in HD are still able to release a sufficient amount of hypocretin to prevent narcoleptic-like symptoms,<sup>10,69,137</sup> which is in accordance with rat studies indicating a need for a 73% decline in neuronal number to decrease CSF hypocretin levels by half.<sup>70</sup> Nevertheless, apart from one study in seven HD patients that found normal CSF hypocretin-1 levels independent of the presence of sleep disorders and nutritional status,<sup>10</sup> CSF hypocretin levels have not yet been investigated more accurately in conjunction with neuroendocrine and clinical parameters, particularly those concerned with sleep, ANS function and body weight and its composition. Thus, at present, any (sub)clinical consequences of a relative hypocretin deficiency in HD cannot be excluded with certainty. It would be interesting to assess another neuronal population in HD brains that is localized in the lateral hypothalamus, namely the melanin-concentrating hormone (MCH)-producing neurons. Like hypocretin, MCH plays a pivotal role in the orchestration of hypothalamic functions, in particular systemic energy homeostasis<sup>195,220</sup> and, therefore, MCH neuronal loss or dysfunction might also partly explain the relentless weight loss in HD patients.

### 3.2.6. *Corticotropin-releasing hormone*

Kurlan et al. found elevated corticotropin-releasing hormone (CRH) concentrations in the CSF of 56 (30 male and 26 female) nonmedicated patients with early (stages I and II) HD in comparison with a control group of

21 subjects without neurological illness.<sup>116</sup> Patients with HD who had depressive disorders (major depression or dysthymia) did not differ from those without depression with respect to CRH concentration. However, a positive correlation was observed between the severity of *major depression* (but not dysthymia) and CRH concentrations in HD patients. Even though it is assumed that CSF concentrations of CRH generally originate from extra-hypothalamic sources rather than the PVN<sup>207</sup>, three observations nonetheless suggest a hypothalamic origin of the elevated CRH found in the CSF of HD patients.<sup>116</sup> First, the CSF concentration of CRH was positively correlated with the severity of major depression, which could indicate the PVN as the origin of the CRH, since an increase of CRH neuron number, CRH-mRNA, and vasopressin colocalization in CRH neurons in the PVN of depressed patients have been demonstrated.<sup>176,177</sup> Second, various studies have found increased plasma levels of both ACTH and cortisol, indicating HPA axis hyperactivity in HD (see below under ‘Endocrine abnormalities in HD’). And third, De Souza et al.<sup>49</sup> measured CRH-like immunoreactivity in control and HD brain tissues obtained post-mortem and found that the concentration of CRH-like immunoreactivity was markedly *decreased* in the caudate/putamen, while no significant differences were observed in the concentrations of CRH-like immunoreactivity between controls and HD patients in frontal, parietal, temporal, occipital and cingulate cortex and in globus pallidus, which also argues against an extra-hypothalamic source of the increased CRH concentration in the CSF of HD subjects<sup>116</sup>; although it should be noted that there are also other sources of extra-hypothalamic CRH such as the thalamus.<sup>8</sup> Assuming a hypothalamic origin of the elevated liquor CRH in HD, two questions remain to be sorted out: first, what is the cause of this elevation, and second, what are exactly the functional consequences of increased CRH production in the hypothalamus of HD patients? Regarding the first question, Heuser et al.<sup>79</sup> suggested that the endogenous CRH overdrive in HD subjects might be due to a loss of GABA-containing medium-spiny neurons in the striatum of patients with HD. Interestingly, experimental data in the rat shows that loss of GABA-ergic innervation per se can independently add to the anorexigenic actions of excess CRH as discussed below.<sup>92</sup> However, administration of muscimol, a potent GABA-agonist, seemed to have no effects on plasma cortisol levels in 4 HD patients,<sup>209</sup> rendering the explanation offered by Heuser et al.<sup>79</sup> somewhat questionable. With regard to the second question, CRH is the primary hypothalamic hormone stimulating the release of pituitary ACTH, which stimulates cortisol secretion from the adrenal glands. CRH-producing cells involved in regulation of the pituitary-adrenal axis are localized mainly in the parvocellular subdivision of the PVN.<sup>92</sup> Central injection of CRH produces behavioral effects suggestive of major depression as well as anorexia, as evidenced by attenuation of nocturnal and fasting-induced feeding, and diminishes feeding in a number of pharmacological and behavioral paradigms designed to evaluate ingestive behavior in rodents.<sup>92,208</sup> Microinjection studies imply that the site of anorectic action of CRH lies within the PVN, and that CRH, if released locally in the PVN, may tonically restrain the action of endogenous orexigenic signals such as NPY.<sup>92</sup> Heinrichs et al.<sup>78</sup> reported that increased availability of CRH/urocortin in the hypothalamus by the chronic central infusion of rat/human CRH(6–33), a high affinity CRH binding protein inhibitor, significantly decreased body weight in Zucker obese rats that normally have reduced CRH stores in the hypothalamus. However, hyperphagia was unabated in these animals, and the investigators suggested that the loss in body weight was due to CRH/urocortin-induced increase in energy expenditure and sympathetic tone produced by thermogenesis and lipolysis<sup>78,92</sup> since CRH neurons from the PVN project extensively to several autonomic centres in the brain stem. Thus likewise, a chronic CRH overdrive in the PVN of HD subjects may substantially contribute to the emaciation seen in these patients, who nevertheless, frequently report an insatiable appetite. Although it should be noted that, apart from the physiological stimulus caused by a protracted state of negative energy balance, increased appetite in HD patients may, at the very least, also originate from direct pathology and/or dysfunction of the nucleus accumbens<sup>11,24,65,182</sup> and alterations



of its enkephalinergetic input from the more dorsal domains of the striatum.<sup>193</sup> This is because the hedonic experience of the feeding consummatory act, particularly with regard to palatable foods, is largely mediated by opioid transmission throughout both the ventral (i.e. nucleus accumbens) and dorsal striatum<sup>96</sup> and the opioid-peptide containing medium-sized spiny neurons of the (particularly dorsal) striatum are among the neuronal groups preferentially affected in HD.<sup>138,193</sup> Altered dopaminergic transmission through the nucleus accumbens could also play a role.<sup>96</sup>

### 3.2.7. *α-Melanocyte-stimulating hormone*

Konagaya and Konagaya<sup>105</sup> measured  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH)-like-immunoreactivity in the CSF of, among others, 2 patients with HD. As compared to the values measured in control subjects, the  $\alpha$ -MSH immunoreactivity in HD patients was not deviant.  $\alpha$ -MSH is, along with ACTH, a peptide cleavage product of the proopiomelanocortin (POMC) molecule and the main endogenous ligand of the melanocortin-3 and melanocortin-4 receptors which when stimulated suppress food intake. Levels of POMC mRNA are normally markedly reduced in fasted animals and increased by exogenous administration of leptin.<sup>236</sup> Leptin increases melanocortin signalling, both by increasing  $\alpha$ -MSH release from POMC neurons and by decreasing production of the melanocortin receptor antagonist agouti-related protein (AGRP).<sup>233</sup> As HD patients are often underweight<sup>52</sup> and as plasma leptin concentrations appear to be decreased in HD patients,<sup>171</sup>  $\alpha$ -MSH values within the control range<sup>105</sup> seem to be paradoxically normal in this group. In addition, melanocortin signalling in the hypothalamus appears to be involved in the pathogenesis of the anorexia and weight loss associated with inflammatory and neoplastic disease processes.<sup>234</sup> Although the precise mechanism whereby peripheral inflammatory/neoplastic factors activate the melanocortin system remains unknown, the proinflammatory cytokines (interleukin-1, interleukin-6, and tumour necrosis factor- $\alpha$ ) that are produced in the hypothalamus during both inflammatory and neoplastic disease processes are likely to play a role.<sup>233</sup> Interestingly, activation of various immune system compartments in HD have been reported as well.<sup>123</sup> Thus, alterations in the hypothalamic melanocortin system may also bear a part in the pathogenesis of wasting in HD.

## 4. ENDOCRINE ABNORMALITIES IN HD

For a discussion of alterations in hypothalamic-releasing/-inhibiting factors in HD patients please refer to the previous section. In the following section the functional integrity of the hypothalamo-pituitary-target organ axes will be dealt with.

### 4.1. Hypothalamo-neurohypophysial system

In order to assess the functionality of the hypothalamo-neurohypophysial system Lavin et al.<sup>120</sup> performed a water deprivation test in a group of eight HD patients: the patients seemed to have retained their ability to concentrate urine which suggests intact osmoreceptor function and sufficient vasopressin release from the posterior lobe of the pituitary in HD. However, hypothalamic/neurohypophysial vasopressin and oxytocin

contents have never been evaluated in HD brains. Apart from their neuroendocrine functions, both vasopressin and oxytocin appear to have central neuromodulatory roles as well, with effects on, among others, blood pressure, thermoregulation, food intake, cognition, mood and hypothalamo-pituitary-adrenal axis,<sup>207</sup> all of which are domains that may be affected to some degree in patients with HD. Post-mortem amounts of vasopressin immunoreactivity in the locus ceruleus and substantia nigra of 10 cases of HD were comparable to those of normal controls.<sup>183</sup> As in the human brain the vasopressin immunoreactivity in these areas is confined to fibers originating in the hypothalamus, in all probability the PVN,<sup>219,225</sup> the latter findings would argue for an intact hypothalamic vasopressinergic innervation. It is not known, however, to what extent disturbed central vasopressin synthesis and/or release could account for orthostatic hypotension in some HD patients. No data are available with respect to oxytocin in HD patients. Although parturition and lactation difficulties are not noticeably common in the past history of female HD subjects, this does not exclude abnormalities in oxytocin production and/or release during the illness since the disease often commences after the usual age of childbirth.

As described before, cell degeneration and gliosis have been reported in HD subjects in both the SON and PVN,<sup>188,227</sup> which are the production sites of pituitary vasopressin and oxytocin, and in a recent study in a mouse model of HD the hypothalamic expression of the mRNAs of both vasopressin and oxytocin were shown to be significantly down-regulated.<sup>107</sup> This creates precedent for further exploration of this area in HD patients.

## 4.2. Hypothalamo-pituitary-adrenal (HPA) axis

Originally, Bruyn et al.<sup>31</sup> reported decreased plasma levels of cortisol in some patients with HD, but later studies by others consistently indicate hyperfunction, instead of hypofunction, of the HPA axis in HD. CSF levels of CRH in HD individuals have been reported to be increased (see above under the subheading ‘CRH’ for a discussion of this finding). Basal levels of both ACTH<sup>79</sup> and cortisol<sup>79,122</sup> seem to be increased as well. However, after an intravenous CRH challenge test, ACTH responses tended to be blunted in concert with normal cortisol levels, indicating that the negative feedback regulation of glucocorticoid secretion is probably intact at the pituitary-adrenal level.<sup>79</sup> Following an overnight dexamethason suppression test, the mean cortisol levels did not differ significantly between the HD and the control group, whereas mean plasma dexamethason concentration was 57 % lower in patients than in controls,<sup>79</sup> although the precise cause and significance of this latter finding remains unclear. Moreover, the cortisol (and GH) rise during hypoglycaemia, induced by intravenous insulin administration, occurred earlier in HD patients, though peak responses were the same in each group.<sup>120</sup> Durso et al.<sup>56</sup> found no significant differences in mean plasma cortisol concentrations between their patient and control samples, though it should be noted that their samples consisted exclusively of female subjects and that three of the nine participating HD patients had the rigid-akinetic variant of the disease whose response may be diametrically opposed to that of choreic patients.<sup>75</sup> On the other hand, Leblhuber et al. found significantly higher basal plasma levels of cortisol in 11 drug-free male HD patients compared with age- and sex-matched normals.<sup>122</sup> In addition, in a recent study Björkqvist et al. reported progressive alterations in the HPA axis in the R6/2 mouse reminiscent of a Cushing-like syndrome.<sup>20</sup> The R6/2 mice showed enlargement of the intermediate lobe of the pituitary, hypertrophy of the adrenal cortex, increased ACTH plasma concentrations as well as a progressive increase in serum and urine corticosterone levels. However, the hypothalamic CRH levels were reduced by 62 % in the R6/2 mice suggesting that CRH released from the hypothalamus is not driving

the pituitary ACTH production in these mice, which appears to be at odds with the available data on CRH in humans with HD.<sup>116</sup> Normally dopamine represses ACTH expression, but in the R6/2 mice the expression of pituitary dopamine D2 receptors was reduced by half which therefore could explain the increase in ACTH levels in these mice.<sup>20</sup> Björkqvist et al. corroborated some of their findings in the human disease by measuring cortisol contents in urinary samples from 82 HD patients and 68 controls: the cortisol levels were significantly elevated in moderate (stage III) and moderate-advanced stage (stage IV) patients, whereas pre-symptomatic and early disease stage (stage I/II) patients exhibited levels that were not significantly different from age- and sex-matched controls. It is important to note that the R6/2 mice also exhibited muscular atrophy, reduced bone mineral density, abdominal fat accumulation and insulin resistance, all of which could be consequences of increased glucocorticoid levels. Therefore, elevated levels of cortisol may also contribute to the clinical symptoms like muscular wasting, mood changes and some of the cognitive deficits that occur in patients with HD.<sup>20</sup>

Although Bruyn et al.<sup>30</sup> could not detect any changes in serum levels of dehydroepiandrosterone sulphate (DHEAS), the major androgen secreted by the adrenals, Leblhuber et al.<sup>122</sup> found, apart from higher basal levels of cortisol, significantly lower serum levels of DHEAS; consequently, there was also a significant difference between the DHEAS/cortisol ratio in their index and reference group which is in line with some previous findings.<sup>150</sup> Because DHEAS is known to reduce the levels of glucocorticoids as well as antagonize their functions, DHEAS suppletion could be a strategy to counteract the noxious effects of prolonged hypercortisolaemia on e.g. cognition, mood and metabolism in HD subjects.<sup>121,122,150</sup>

### 4.3. Somatotropic axis

Alterations in GH secretion in HD have frequently been reported and often entail an increase in *mean* basal plasma GH levels and exaggerated or paradoxical responses of GH secretion during stimulatory and/or inhibitory functional tests.<sup>98</sup> In contrast, most authors have found non-deviant GH concentrations during *single* baseline measurements.<sup>17</sup> However, exaggerated GH responses followed administration of L-dopa<sup>167</sup>, apomorphine<sup>55</sup>, bromocriptine<sup>35</sup>, arginine<sup>125,168</sup>, insulin<sup>97,120,165</sup> and muscimol<sup>55</sup>. Paradoxical GH responses have been observed to occur after administration of glucose<sup>51,115,166,167</sup>, L-dopa<sup>51</sup> or bromocriptine.<sup>36,51</sup> It should be noted though that exaggerated and paradoxical GH responses were not always uniformly present, i.e. while some HD patients exhibited an exaggerated response during a particular functional test, others exhibited a normal or even paradoxical response,<sup>51</sup> and not all authors have been able to detect significant abnormalities in GH secretion,<sup>47,115,190</sup> either suggesting the existence of considerable heterogeneity among individual HD patients in this respect and/or reflecting different methods in performing and interpreting the endocrine tests or, alternatively, differences in the severity of the disease.<sup>115</sup> Interestingly, Hayden et al. found that a dose of 25 mg of chlorpromazine suppressed GH release in adults with HD, but not in controls, perhaps indicating dopamine receptor hypersensitivity in the HD group.<sup>75</sup> Although Durso et al.<sup>56</sup> and Murri et al.<sup>144</sup> have evaluated, respectively, basal 24-hour and nocturnal plasma levels of GH in HD, their long blood sampling interval (i.e. 30-minutes or more) does not allow for definite judgments regarding the precise secretory pattern of GH release in HD, and any possible alteration therein, because of the fairly short half-life of this hormone in plasma.<sup>226</sup> Kremer et al.<sup>115</sup> measured plasma levels of insulin-like growth factor-1 (IGF-1), an important

peripheral mediator of GH activity with a comparatively long half-life, in 10 HD patients at baseline and in 8 of them also after a 2.5-year follow-up, but found no significant differences, either between the two intra-individual measurement points or in comparison to values in matched controls; yet the authors stated that the assay applied probably has not been sufficiently accurate in detecting small differences.<sup>115</sup> Non-deviant basal IGF-1 levels were also reported by other authors.<sup>171</sup> Since the nature of impaired GH regulation in (some) patients with HD does not imply hypophysiotropic pathology, deviant GH release in HD might be attributed to impaired input from structures elsewhere (H.P. Kremer, thesis). GH has long been known to have profound effects on systemic substrate metabolism. Prolonged elevated levels of GH can induce glucose-intolerance and insulin-insensitivity as is the case in acromegalics. On the other hand GH is a potent physiological stimulator of lipolysis and e.g. GH suppletion can result in considerable loss of body fat and weight in (adult) patients with Prader-Willi, the most common syndromal cause of morbid obesity.<sup>81</sup> Therefore, the abnormalities in carbohydrate metabolism in HD (see below under the section ‘Metabolic abnormalities in HD’) and wasting in this disorder could both be attributed, at least in part, to hypersomatotropism in some of these patients.

#### **4.4. Lactotropic axis**

Many studies have targeted lactotropic axis function in HD patients. In general, prolactin dynamics are considered to be normal or slightly increased.<sup>98</sup> Most studies report normal plasma prolactin concentrations during single daytime<sup>35,36,120,126,142</sup>, nocturnal<sup>144</sup> or 24-hour determinations.<sup>56</sup> Although a few authors have described lower<sup>76,158</sup> or increased basal levels<sup>33,35</sup>, in these studies an insufficient wash-out period of previous neuroleptic medication may have confounded the results.<sup>98</sup> Normal suppression of plasma prolactin followed dopaminergic stimulation<sup>35,36,55,126,143</sup> and the prolactin response to muscimol (a GABA-agonist) as well as arecoline (a muscarinic agonist) were found to be similar in the HD and the control group.<sup>55</sup> Initially, blunted prolactin responses were reported after chlorpromazine or TRH stimulation,<sup>33,76</sup> but subsequent studies could not confirm these findings.<sup>35,120,126</sup> Considering the methodological shortcomings or strengths of the various studies, the evidence from these early findings favours a slight increase in plasma prolactin levels.<sup>98</sup> Interestingly, Kremer et al.<sup>115</sup> reported a decrease in the mean basal prolactin levels over 2.5 years of follow-up in 7 patients with HD (though it should be noted that at baseline the prolactin levels of their patients and controls did not differ and that the control subjects could not be reinvestigated after the follow-up period), which clearly contradicts earlier findings.<sup>98</sup> It remains to be sorted out to what extent this should be attributed to seasonal fluctuations,<sup>115</sup> a statistical type I error<sup>115</sup> or be considered as evidence for a ‘hyperdopaminergic state’ in HD as proposed in the earliest report by Hayden et al.<sup>76</sup> Interestingly, more recent experimentation indeed hints at an important role for altered dopaminergic signaling in the striatal vulnerability associated with HD,<sup>37,87</sup> although the implications for the hypothalamic tubero-infundibular dopaminergic system and its regulation of prolactin release are still obscure.

#### **4.4. Hypothalamo-pituitary-thyroid (HPT) axis**

Relatively few studies in HD patients have evaluated HPT axis activity. Basal levels of total thyroxine, triiodothyronine, triiodothyronine resin uptake and TSH have been reported to be comparable to values in normal controls.<sup>76,120</sup> Lavin et al.<sup>120</sup> found that the TRH test revealed no differences in basal levels of TSH,

or in peak response to TRH or in the increment at 20 minutes, although one of the 8 patients, but none of the 10 controls, had a delayed response typical of a hypothalamic disorder.<sup>120</sup> However, Hayden et al.<sup>75</sup> reported impaired TSH response to TRH stimulation in 7 adults with HD as compared to 11 normal controls. Since depression is very common in HD and TRH stimulation test reveals a lower or blunted TSH response to TRH in a substantial proportion of depressed patients as well,<sup>59</sup> it remains unclear to what extent the findings by Hayden et al.<sup>75</sup> in fact might have reflected a concomitant depressive disorder in the patient group. Importantly, neither Lavin et al.<sup>120</sup> nor Hayden et al.<sup>75,76</sup> reported the mean body weight and the overall nutritional status of their subjects. Since it is well known that levels of thyroid hormones drop during fasting or negative energy balance<sup>90</sup> and as many HD patients are underweight, values of T3, T4, fT4 and TSH within the 'normal' range in this group could, nonetheless, be indicative of HPT axis dysfunction. It is in this regard of interest to note that in a retrospective chart review study of 97 HD patients residing in long-term care facilities, the most commonly prescribed drug (6%) for problems 'unrelated' to HD was found to be levothyroxine.<sup>146</sup> Moreover, both leptin and glucocorticoids appear to exert substantial modulatory effects on the thyroid axis<sup>90,170</sup> and as there are indications that both hypoleptinaemia and hypercortisolaemia may be features of at least some patients with HD (see above under the headings 'Ghrelin and leptin' and 'Hypothalamo-pituitary-adrenal (HPA) axis'), it can be argued that closer examination of the HPT axis function in this disorder may demonstrate abnormalities, which would be of particular relevance for elucidating the pathogenesis of wasting and mood changes in this disease.

#### 4.6. Hypothalamo-pituitary-gonadal (HPG) axis

For a review of HPG axis function in HD refer to the subheading 'Gonadorelin' in the section 'Hypothalamic dysfunction in HD'.

#### 4.7. Ghrelin and leptin

Ghrelin, an orexigenic factor of gastric origin, and leptin, a peptide hormone secreted by adipose tissue, are two peripherally produced hormones that exert opposite effects on the neuronal populations within the arcuate nucleus, VMN and lateral hypothalamus that play a key role in the regulation of body energy homeostasis.<sup>236</sup> Popovic et al.<sup>171</sup> measured both circulating and CSF levels of ghrelin and leptin in 15 patients with HD and 20 normal-weight subjects undergoing orthopaedic surgery. Blood samples were obtained by venipuncture and in-parallel CSF samples for ghrelin and leptin determination were obtained by lumbar puncture. Patients with HD had increased concentrations of ghrelin in plasma compared with healthy subjects ( $4523.7 \pm 563.9$  vs.  $2781.1 \pm 306.2$  pg/ml,  $P < 0.01$ ). On the other hand, patients with HD had decreased concentrations of leptin in plasma compared with healthy subjects ( $4.8 \pm 1.6$  vs.  $10.9 \pm 2.4$  ng/ml,  $P < 0.01$ ). Comparably, in the CSF, the concentrations of ghrelin tended to be higher and the levels of leptin tended to be lower, but these differences failed to reach statistical significance, possibly due to the relatively small sample size.<sup>171</sup> It should be noted though that the index and the reference group were not matched for BMI ( $20.2 \pm 0.6$  vs.  $24.8 \pm 0.8$  respectively,  $p = 0.001$ ) and that the total fat mass was not calculated and as a result the circulating leptin levels were not corrected for total fat mass. Nevertheless, *only* in the control group did plasma leptin concentration correlate with BMI. Two other studies reported equivalent plasma leptin levels in HD patients and controls matched for



BMI, fat mass and fat-free mass.<sup>68,172</sup> However, the patients in these studies were, by selection, asymptomatic or were at a mild to moderate stage of the disease (stages I and II). Other confounders which could account for the discrepancy between the findings in these studies are: a) the circadian rhythm of plasma leptin levels, which are (like ghrelin levels) high in the morning and low at night;<sup>236</sup> b) plasma levels of cortisol and insulin,<sup>45</sup> both of which could be deranged in HD patients (refer to the subheadings ‘Hypothalamo-pituitary-adrenal (HPA) axis’ and ‘Carbohydrate metabolism’ for further details) and c) differences in sympathetic tone,<sup>102</sup> which as mentioned before, may vary in the course of HD. Furthermore, it is not known to what extent increased levels of circulating ghrelin, which is the native substrate for the GH-releasing peptide (GHRP) receptor, could underlie the hypersomatotropism in some HD patients (see under the subheading ‘Somatotropic axis’ below), as high GH responses are induced by ghrelin infusions which act synergistically to growth-hormone releasing hormone (GHRH) stimuli.<sup>101,163</sup>

## 5. SYSTEMIC METABOLIC ABNORMALITIES IN HD

In the following section we will give an overview of alterations in carbohydrate, lipid and protein metabolism in HD as well as their relation to the afore discussed hypothalamic and endocrine alterations and clinical symptomatology.

### 5.1. Carbohydrate metabolism

The studies on carbohydrate metabolism in HD subjects have yielded quite ambiguous and conflicting results.<sup>115</sup> Considerably large groups of HD patients have been identified with impaired glucose tolerance on oral glucose loading,<sup>51,168,169,190</sup> although none of the participants displayed clinical signs or symptoms of diabetes mellitus. These studies compared the findings in individual patients with a pre-chosen standard, which was lower than currently used<sup>168,169</sup> or ill-defined.<sup>51</sup> Remarkably, other investigators identified appreciably fewer HD patients with impaired glucose tolerance.<sup>47,115</sup> Though it should be emphasized that generally, in the earlier studies, information about the precise clinical condition (especially body weight and composition, stage of the disease, medication and immobility status) of the participants is lacking and the results of individual patients are not shown and therefore cannot be reinterpreted according to the current WHO-criteria.<sup>115</sup> Several authors have also performed an analysis of their pooled data on the oral glucose tolerance tests by comparing the mean values of HD patients with those of control subjects: only Podolsky et al.<sup>168,169</sup> were able to detect significant differences, while others could not replicate their findings.<sup>47,97,115,166</sup> In addition, Kremer et al. reported glycosylated haemoglobin (HbA1c) levels in their HD patients to be comparable to those of controls, excluding marked blood glucose fluctuations over the preceding three to four weeks in their sample.<sup>115</sup> Elevated levels of circulating insulin were noted in all patients with impaired glucose tolerance,<sup>168,169</sup> whereas other studies reported mean insulin levels during glucose tolerance testing to be normal and fasting glucose,<sup>68,172,216</sup> insulin and C-peptide levels to be unchanged.<sup>47,97,115,115</sup> Despite the increased insulin levels found by some authors, insulin tolerance tests in HD patients did not demonstrate any abnormalities.<sup>97,120,166</sup> Of note is that during the insulin tolerance tests all patients, although awake, lost their involuntary movements;<sup>97,120</sup> in Lavin’s group the disappearance of the movements coincided with the onset of hypoglycaemia: a possible explanation could be

that the basal ganglia in HD are especially susceptible to neuroglycopenia.<sup>120</sup> Only one follow-up study has been performed in which glucose tolerance, HbA1c and insulin values were assessed in HD patients; none of the investigated parameters showed any significant change after 2.5 years, although the drop-out rate was substantial (4 of the original 10 HD patients could not be reinvestigated for the evaluation of glucose tolerance and in 2 of them HbA1c and insulin could not be assessed either).<sup>115</sup>

In a retrospective study by Farrer et al.<sup>61</sup> information about the incidence and control of diabetes mellitus in 620 probands (278 living, 332 deceased) with HD and in their first and second degree relatives was obtained by a questionnaire method. Among the probands, 65 individuals (10.5%) were identified by the informant or verified by examination of the family records as diabetic. The age adjusted prevalence rate for the year 1975 of diabetes in HD was calculated to be 4 times as high as in the general population. Incidence rates were not calculated because of ascertainment and other biases in their data. Interestingly, results from the analysis of family data indicated that HD affected relatives of an HD proband with diabetes were 7 times as likely to have diabetes over the proband's non-HD relatives, while a non-diabetic HD proband was equally likely to have an HD or non-HD relative with diabetes, suggesting genetic clustering of diabetes and HD. It should be noted that unambiguous interpretation of Farrer's findings is hampered by incomplete data on their criteria for diabetes, body composition, the use of drugs, intercurrent illness, and the level of activities, as well as by the intrinsic difficulties associated with a retrospective survey.<sup>115</sup>

More recently, Underwood et al.<sup>218</sup> applied metabolic profiling to serum samples from HD patients (10 asymptomatic gene carriers and 20 patients in the early to moderate stages of their disease) in a non-hypothesis driven systems biology search for disease biomarkers and found, among others, significant changes in various monosaccharide levels (including glucose) between asymptomatic gene carriers/patients on the one hand and 20 controls of similar age and sex distribution on the other hand. However, the authors did not indicate the direction of the changes and whether the blood samples were taken in a fasting state.

In sum, due to principal and/or methodological inadequacies of most of the studies on carbohydrate metabolism in HD patients and the very small sample sizes often examined, which in all probability both could account for the contradictory results, it still remains to be ascertained whether impaired carbohydrate metabolism is an inherent feature of HD and whether it has any special clinical relevance in this disorder (especially with regard to weight loss). If this is the case, the precise underlying mechanisms (i.e. pancreatic  $\beta$ -cell dysfunction,<sup>4,19,82,85</sup> possibly due to transcriptional dysregulation,<sup>4</sup> and/or peripheral insulin insensitivity<sup>20</sup> secondary to neuroendocrine and ANS dysbalance due to hypothalamic dysfunction (see previous sections)) should be elucidated in order to devise effective therapeutic strategies. As recent experimentations in animal models of HD demonstrate that the prevalence of impaired glucose tolerance is consistently higher in the affected animals compared to controls<sup>4,19,20,82,85,88,129,218</sup> and that dietary manipulations that affect glucose metabolism may lead to amelioration of symptoms and/or increase overall life-span in these animals<sup>53</sup> combined with strong indications that lately have emerged for a saccharide-polyglutamine interaction both in vitro and in vivo,<sup>95,210,211</sup> corroboration of these data in humans, in both principally and methodologically sound and rigorous experimental settings, seems to be necessary.

## 5.2. Lipid metabolism

Notwithstanding the progressive weight loss in HD patients, which is accompanied by substantial loss of body fat stores, as indicated by decreased abdominal circumference and subscapular skinfold (both measures of central/visceral adiposity) as well as triceps skinfold thickness (a measure of peripheral adiposity),<sup>62,63,216</sup> *systemic* lipid metabolism has hardly received any direct study in individuals with this disorder. Schubotz et al.<sup>190</sup> measured serum lipids and the plasma fatty acid composition of the cholesterylesters, triglycerides and phospholipids in 25 subjects with or at risk for HD (9 at-risk asymptomatics, 5 with light and 11 with severe symptoms) and found minor deviations in the fatty acid patterns in various lipid classes. Other authors have reported high fasting concentrations of non-esterified fatty acids (NEFAs) in choreic patients when compared with control subjects.<sup>166</sup> This difference was maintained under hypoglycaemic conditions, while during hyperglycaemia the differences in NEFAs concentrations between the groups was abolished.<sup>166</sup> In addition, plasma and CSF leptin concentrations have been measured (refer to the subheading ‘Ghrelin and leptin’ in the section ‘Endocrine abnormalities in HD’ for a discussion of these measurements). Recently, Underwood et al.<sup>218</sup> applied metabolic profiling to serum samples from HD patients (10 asymptomatic gene carriers and 20 patients in the early to moderate stages of the disease) in a non-hypothesis driven systems biology search for disease biomarkers. Among other things, they found significant changes in various markers of fatty acid breakdown (including glycerol and malonate) between asymptomatic gene carriers/patients and controls of similar age and sex. This could indicate a pro-catabolic phenotype early on in the disease progression,<sup>218</sup> although the precise significance of these and previous findings still remains to be clarified. In addition, the possible contribution of hypothalamic dysfunction in HD, and subsequent endocrine abnormalities (especially GH and cortisol) and autonomic dysregulations (in particular a possible dysbalance between sympathetic and parasympathetic innervation of adipose tissue,<sup>108,109</sup> to the genesis of systemic alterations of fat metabolism in HD is far from clear and needs further exploration.

Circumstantial evidence initially obtained from a small series of publications in the 1970s that all claimed to have discovered membrane abnormalities in cells of peripheral tissues (especially fibroblasts and erythrocytes) from HD patients, led to the belief that a ‘generalised cell membrane defect’ may be involved in HD.<sup>15</sup> This prompted intensive efforts by many investigators over a considerable time span to reproduce these initial findings, but most of the later studies failed to substantiate the existence of a ‘generalised membrane defect’ in HD.<sup>15,131,189</sup> In particular, since biochemical findings regarding phospholipid concentration, fatty acid analysis, lipid-bound sialic acid, neutral glycolipids, and total cholesterol were reported to be normal in erythrocyte lipid fractions from HD patients<sup>23,80,230</sup> and as membrane fluidity of HD fibroblast, erythrocyte and lymphocyte membranes did not appear to be significantly different from that of controls,<sup>16,117,205,206</sup> Beverstock<sup>15</sup> concluded that in any event lipid fractions of *peripheral* cell membranes and blood plasma are not involved in HD. However, in a recent study, Valenza et al.<sup>222</sup> extracted RNA from primary fibroblasts taken from control and HD patients and analyzed the expression of three key genes of the cholesterol biosynthetic pathway, viz. HMG-CoA reductase, cytochrome P450 lanosterol 14  $\alpha$ -demethylase and 7-dehydroxy-cholesterol reductase, and found that HD fibroblasts show a 35-40% decrease in the mRNA levels of these three genes. Although they observed no differences in cholesterol biosynthesis between normal cultured fibroblasts and HD fibroblasts in standard medium, exposure of the cells to lipoprotein-deficient serum (LPDS) demonstrated that HD fibroblasts were less capable of upregulating the mevalonate pathway, and thus *de novo* cholesterol synthesis, in response to low levels of cholesterol;<sup>222</sup> this latter finding is in line with that of Menkes et al. who showed that control fibroblasts were not affected unduly by LPDS over a four day period, whereas three-quarters of the



HD lines failed to grow in LPDS.<sup>139</sup> The discrepancy between the recent findings of Valenza et al.<sup>222</sup> and the conclusion drawn by Beverstock,<sup>15</sup> which was based on a review of earlier studies on lipid metabolism in HD, could be accounted for by the possibility that some degree of stressing of cells might be necessary to unveil a phenotypic difference in HD cells. However, in a later study Sakai et al.<sup>185</sup> did find that the mean level of docosahexanoic acid in the erythrocyte membrane in six HD patients was significantly lower than in that of 14 matched controls. Therefore, considerable controversy remains about any putative defects of peripheral tissue lipid metabolism in HD.

Contrary to *peripheral* lipid metabolism, the indications of a defect in *neuronal* lipid metabolism in HD are more consistent. Ellison et al.<sup>57</sup> reported the concentrations of phosphoethanolamine and ethanolamine to be significantly reduced in the caudate, putamen and nucleus accumbens of brain samples from HD subjects. More recently, by applying the niacin skin flush response (i.e. measuring the cutaneous erythematous vasodilatation response to a topical aqueous methyl nicotinate solution) Puri<sup>173</sup> demonstrated that the response in 6 patients with advanced HD (stage III) was significantly lower than the mean response in a group of 14 age- and sex-matched controls, suggesting an abnormality of neuronal membrane fatty acid metabolism, particularly an impaired phospholipid-related signal transduction, in advanced HD.<sup>173</sup> Still more recently it was shown that the transcription of key genes of the cholesterol biosynthesis pathway is severely affected in both human post-mortem HD striatal and cortical tissue<sup>222</sup> as well as in HD animal derived brain tissues.<sup>200,222</sup>

Based on the hypothesis that a membrane defect might be fundamental in HD pathogenesis and that this might be partly corrected by the provision of essential fatty acids,<sup>46</sup> a few open label studies with  $\gamma$ -linolenic acid and eicosapentaenoic acid (EPA) were conducted in patients with HD that showed significant clinical improvement in both motor and cognitive performances.<sup>221</sup> Subsequent small-scale studies showed that treatment with ethyl-EPA in HD is associated with decreased abnormal movements and changes on brain magnetic resonance imaging when compared to a placebo-treated group.<sup>174,221</sup> In a recent large-scale (n = 135) clinical trial on the efficacy of 2 g/d ethyl-EPA versus placebo for the treatment of HD patients,<sup>175</sup> intention-to-treat analysis after 12 months of treatment did not reveal a significant difference between ethyl-EPA and placebo on the primary end point of the study, viz. Total Motor Score 4 subscale (TMS-4) of the UHDRS. However, restriction of the analysis to those patients who had completed the study without protocol violations (n = 83), did indicate ethyl-EPA to be better than placebo as judged by the TMS-4 scores. It should be noted though that intention-to-treat analysis showed a significant worsening of the behavioural score in the EPA-treated group compared with the placebo-treated group.

In conclusion, the studies on lipid metabolism in HD patients hitherto have yielded equivocal results and clearly do not suffice to pass a well-considered judgment on any possible systemic defects in lipid metabolism in these subjects. As recent findings in animal models of HD<sup>5,42,60,223,224</sup> do support the notion of systemic anomalies of lipid metabolism in HD and as supplementation of certain lipid groups could be efficacious in patients with HD,<sup>175,221</sup> further systematic exploration of this field, e.g. by measuring lipid synthesis and/or lipolysis in vivo, could yield interesting results.

### 5.3. Protein metabolism

Using the anthropometric data on upper arm and calf circumferences and triceps and calf skinfold thicknesses

from the studies by Farrer et al.<sup>62,63</sup> in combination with the following formulae: upper arm muscle circumference = upper arm circumference –  $\pi \cdot$  (triceps skinfold thickness) and calf muscle circumference = calf circumference –  $\pi \cdot$  (calf skinfold thickness), it can be calculated that individuals affected with HD have a significantly smaller upper arm as well as calf muscle circumferences compared to controls, despite a comparable protein and fat intake and even a higher carbohydrate intake and less strenuous physical activity.<sup>62</sup> These findings were recently confirmed in a study by Trejo et al. in 25 HD patients and an equally large group of age- and sex-matched controls.<sup>216</sup> Thus apart from fat loss, muscle wasting probably also contributes to the emaciation in HD. In fact, three more recent studies have indeed found abnormal *in vivo* skeletal muscle energy generation in both symptomatic HD subjects and presymptomatic mutation carriers.<sup>106,128,184</sup> These findings indicate that systemic mitochondrial dysfunction is an early and persistent component of the pathophysiology of HD<sup>184</sup> and it is therefore likely that impaired mitochondrial function will partly underlie muscle wasting in subjects with HD. In addition, microarray profiling of gene expression in skeletal muscle biopsies from HD patients and controls demonstrated distinct expression profiles;<sup>204</sup> none of the biopsy donors had frank diabetes or was emaciated, so it is unlikely that HD muscle gene expression changes would be caused by diabetes or weight loss. Corroboration of these data in a HD mouse model indicated that although both the diabetic phenotype and weight loss in these mice partly contributed to the observed changes in muscle gene expression, neither could explain the complete set of alterations.<sup>204</sup> These data suggest a primary, local biochemical defect in HD muscles (see also<sup>180</sup>), although other factors, such as endocrine abnormalities and aberrant signalling from the central nervous system, are likely to be involved as well.

While the earliest studies of amino acid metabolism in subjects with HD could not detect any appreciable abnormalities,<sup>27,43,152</sup> subsequent investigations have found significant differences in plasma concentrations of certain amino acids.<sup>153,154,159,160,166,179,218,237</sup> The most consistent finding appears to be a decrease in the plasma concentrations of neutral amino acids (especially alanine, valine, leucine and isoleucine) compared to healthy controls. Interestingly, in a recent non-hypothesis driven experimental approach, Underwood et al.<sup>218</sup> found decreased levels of valine in asymptomatic HD-gene carriers as well as symptomatic patients, while the levels of alanine and leucine were elevated in the group of asymptomatic gene-carriers and tended to be lower in more symptomatic patients, suggesting a negative correlation between these metabolites and disease progression.<sup>218</sup> Also considerable systemic alterations in the kinetics of the kynurenine pathway (a major route accounting for the metabolism of over 90% of the non-protein tryptophan in most tissues) have been reported in patients with advanced HD,<sup>202</sup> extending the earlier findings of abnormal tryptophan metabolism in the central nervous system of HD subjects (see<sup>202</sup> and the references therein). However, baseline blood levels of melatonin, also a product of tryptophan metabolism, as well as the rise in melatonin levels after tryptophan loading were recently reported not to be different between HD patients and healthy controls.<sup>40</sup> Notably, a case report has described the stabilization of a HD patient by a low tryptophan diet.<sup>157</sup>

Concluding, as yet it is poorly understood to what extent the aforementioned alterations in protein metabolism are due to local changes (especially peripheral mitochondrial dysfunction (see below under ‘Basal metabolism and energy expenditure’)), and to what extent endocrine alterations (including impaired insulin secretion) or ANS dysregulation could account for these abnormalities.

#### 5.4. Basal metabolism and energy expenditure

The first study on basal metabolic rate (BMR) in HD subjects found that the BMR was markedly increased in a number of patients.<sup>17</sup> However, a subsequent study reported that the BMR, as measured by oxygen consumption in a resting state, was not greater in HD subjects (n = 41; stages I and II) compared with the spouses (n = 22) who did not differ from the patients in regard to age, height or lean body mass (LBM); although unexplained weight loss exceeding 3 kg in the past years was reported by 43% of the HD patients but only by 9% of the spouses.<sup>197</sup> More recently, the BMR and energy expenditure have been investigated more thoroughly in HD patients.<sup>68,172</sup> Prately et al. measured sleeping metabolic rate (SMR) and 24-hour sedentary energy expenditure (24-h EE) in a human respiratory chamber in 17 patients with mild to moderate HD (3 of whom were asymptomatic) and 17 control subjects matched for age, sex, BMI and fat mass.<sup>172</sup> They did not find any differences in SMR between the two groups, but the 24-h EE was approximately 14% higher in patients than in controls and the patients seemed to be in a state of negative energy balance. The increase in 24-h EE appeared to be the result of increased spontaneous physical activity (as measured by radar in the chamber and as reflected in the ratio of 24-h EE to SMR) that was proportional to the severity of the patients' chorea score. However, the increase in 24-h EE did not translate into an increase in total energy expenditure (TEE) measured during 7 days in free-living conditions by using the doubly labelled water technique, apparently because patients with HD engage in less voluntary physical activity.<sup>172</sup> Unfortunately, because not all food records were completed, the authors could not assess dietary intake in most of the subjects during free-living conditions, and therefore, energy balance during this period cannot be judged. Gaba et al. assessed SMR, waking metabolic rate (WMR) and 24-h EE (via indirect calorimetry in a human respiratory chamber) in 13 subjects with HD (stages I and II) and 9 controls matched for age, sex, BMI and body fat percentage.<sup>68</sup> They found a tendency for higher SMR and 24-h EE in HD patients compared to controls, although the differences nearly failed to reach statistical significance, probably owing to the small group sizes. The WMR was, nevertheless, significantly higher in patients and was related to a significantly greater displacement of the centre of mass by HD subjects on a force platform (a measure of physical activity), in all probability as a result of the involuntary movements in the choreic subjects.<sup>68</sup>

Although the findings of a similar resting metabolic rate and SMR in patients and matched controls<sup>172,197</sup> argue against an intrinsic metabolic defect in whole body energy metabolism in subjects with HD, other reports of significant and nearly significant increases in BMR<sup>17,17,68</sup> in HD are more consistent with the possibility of an overall defect in mitochondrial oxidative phosphorylation, as suggested by studies in which the phosphocreatine to inorganic phosphate ratios were measured and shown to be decreased in both presymptomatic and symptomatic subjects with HD.<sup>106,128,184</sup> An extensive review of the evidence in favour of peripheral defects in mitochondrial energy generation in both animals and humans with HD is given by Browne and Beal.<sup>26</sup> While the findings of significantly elevated 24-h EE and WMR in HD subjects, which both correlated with the chorea score, do indicate that *involuntary* movements can contribute to a considerable extent to the energy loss in these patients,<sup>68,172</sup> the fact that the total free-living energy expenditure is the same for patients and matched controls, as a consequence of less *voluntary* physical activity by the patients,<sup>172</sup> does not explain the lower body weight of HD patients despite their normal or even increased appetites. Moreover, the greatest rate of body weight loss appears to be in the final hypokinetic stages of the disease when involuntary movements are far less prominent.<sup>187</sup> However, as pointed out by Gaba et al., it might be that the variability in food intake increases as the total functional capacity (TFC) decreases and as a consequence the higher intakes reported on some

days may not be enough to offset the lower energy intakes reported on other days.<sup>68</sup> All together, the precise contribution of altered BMR and TEE to weight loss in patients with HD still remains elusive. In addition, endocrine parameters and ANS function have not yet been assessed in conjunction with measures of energy intake and/or expenditure in individuals with HD and consequently their precise roles in the emaciation in this group of patients also await further elucidation.

## 6. CONCLUSIONS

Since the hypothalamus is a major control centre in the brain for the regulation of body energy homeostasis, sleep-wake cycles and the coordination of autonomic functions [89,92,232,236], we postulated that hypothalamic dysfunction per se as well as subsequent (neuro)endocrine and metabolic abnormalities in patients with HD may substantially contribute to the pathogenesis of weight loss, sleep disturbances and ANS dysfunction in HD. Moreover, some mood and cognitive disturbances associated with HD may also partially originate from hypothalamic involvement in this disorder. As reviewed, many studies in both animal models and human patients with HD indeed strongly indicate that hypothalamic dysfunction, (neuro)endocrine and metabolic abnormalities may be consistent as well as important features of HD, which could at least partly account for the pathogenesis of the aforementioned signs and symptoms. However, as noted before, the dyskinesia per se as well as peripheral tissue anomalies are in all likelihood also implicated in the pathogenesis of symptoms such as unintended loss of body weight in this disorder.

Emaciation, sleep disturbances and autonomic dysfunction are important indicators of the rate of disease progression and can considerably impair the already diminished quality of a patient's life. It is therefore of crucial and practical importance to elucidate the pathophysiology of these signs and symptoms in order to find novel and more effective therapeutic targets. On the basis of this review, it can be inferred that therapies aimed at affecting hypothalamic, (neuro)endocrine and metabolic parameters may indeed be promising potential candidate treatments for the control of these signs and symptoms that should be put to the test in future animal studies and subsequently in human clinical trials as well.

## 7. AIMS OF THE THESIS

### *Part I: Secondary signs in Huntington's disease*

In the first part of the thesis, we aim to delineate of the characteristics of the less well-known symptoms and signs of HD, and assess their association with other aspects of the disease, including mutation size and motor, cognitive and behavioural indices. The course of weight loss and its determinants is assessed in **Chapter 2**. Guided by findings from the previous chapter, in **Chapter 3** the effects the interaction between mutant and normal *HTT* on clinical phenotype, including body weight, are described. Subsequently, the prevalence, nature and correlates of sleep disturbances (**Chapter 4**) and autonomic complaints (**Chapter 5**) in HD patients are

presented.

### *Part II: Hypothalamic pathology in Huntington's disease*

The subject of this part of the thesis (**Chapter 6**) is the neuropathological evaluation of hypocretin-1 (also known as orexin-A) and melanin-concentrating hormone neurotransmission in HD patients, as well as the assessment of various hypothalamic regions for the presence of neuronal intranuclear and cytoplasmic inclusions of mutant huntingtin.

### *Part III: Endocrine studies in Huntington's disease*

This part of the thesis contains a compilation of a number of endocrine studies in early stage HD patients. The objective is to investigate whether the corticotropic (**Chapter 7**), somatotropic (**Chapter 8**), thyrotropic and lactotropic axes (**Chapter 9**) function, and adipokine (**Chapter 10**) and melatonin (**Chapter 11**) secretion are altered in HD patients compared with matched control subjects.

### *Part IV: Metabolic studies in Huntington's disease*

The studies detailed in this section (**Chapter 12**) are aimed at the evaluation of the systemic metabolism in a group of early stage HD patients, focusing on basal energy expenditure, and glucose and fat metabolism.

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## PART I

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# **S**ECONDARY SIGNS OF **H**UNTINGTON'S DISEASE





*“Symptoms then, are in reality nothing but the cry from suffering organs.”*

**Jean-Martin Charcot** *Leçons cliniques sur les maladies des vieillards et les maladies chroniques.* Paris, 1868.



### **Weight loss in Huntington's disease increases with higher CAG repeat number**

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## ABSTRACT

*Objective:* Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expanded number of CAG repeats in the *huntingtin* gene. A hallmark of HD is unintended weight loss, the cause of which is unknown. In order to elucidate the underlying mechanisms of weight loss in HD, we studied its relation to other disease characteristics including motor, cognitive and behavioral disturbances and CAG repeat number. *Methods:* In 517 early-stage HD patients, we applied mixed-effects model analyses to correlate weight changes over three years to CAG repeat number and various components of the Unified Huntington's Disease Rating Scale (UHDRS). We also assessed the relation between CAG repeat number and body weight and caloric intake in the R6/2 mouse model of HD. *Results:* In HD patients mean body mass index decreased with -0.15 units per year ( $p < 0.001$ ). However, no single UHDRS component, including motor, cognitive and behavioral scores, was independently associated with the rate of weight loss. HD patients with a higher CAG repeat number had a faster rate of weight loss. Similarly, R6/2 mice with a larger CAG repeat length had a lower body weight, whereas caloric intake increased with larger CAG repeat length. *Conclusions:* Weight loss in HD is directly linked to CAG repeat length and is likely to result from a hypermetabolic state. Other signs and symptoms of HD are unlikely to contribute to weight loss in early disease stages. Elucidation of the responsible mechanisms could lead to effective energy-based therapeutics.



Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder caused by an expanded number of CAG repeats in the *huntingtin* gene.<sup>1</sup> It is characterized by motor disturbances, cognitive decline and behavioral problems.<sup>2</sup> Unintended weight loss is also a hallmark of the disease, both in HD patients<sup>3-6</sup> and several transgenic mouse models of HD<sup>7</sup>. Weight loss frequently leads to general weakening and a decline in the quality of life of HD patients.<sup>8</sup> On the other hand, a higher Body Mass Index (BMI) has been associated with a slower rate of disease progression.<sup>9</sup>

The cause of weight loss in HD is unknown. It might result from decreased caloric intake, increased motor activity or a higher metabolic rate.<sup>10</sup> Previous studies in both HD patients and transgenic mouse models of HD have shown that loss of weight occurs despite adequate or even increased caloric intake.<sup>11-13</sup> Weight loss is already manifest in presymptomatic HD gene carriers<sup>14</sup> and is particularly marked in the final hypokinetic stages of the disease<sup>5</sup>. These observations and recent findings in HD transgenic mice suggest that weight loss might be due to an increased metabolic rate.<sup>13,15,16</sup> Other reports suggest, however, that loss of body weight is secondary to a higher sedentary energy expenditure due to unwanted movements.<sup>17-20</sup> Thus, studies on the mechanisms underlying weight loss in HD patients have yielded conflicting results and are inconclusive.<sup>10</sup> The different outcomes of these studies are likely to be due to small group sizes and their cross-sectional nature.

Interestingly, the direct relation between the number of CAG repeats in the mutant *huntingtin* gene and weight loss has not been assessed before. Mutant *huntingtin* could interfere with mitochondrial function in peripheral tissues in a CAG repeat length-dependent manner.<sup>21,22</sup> Consequently, CAG repeat length may predict the extent of systemic energy defects in HD patients.

In this study we therefore aimed to 1) specify the course of weight loss in a large, homogenous group of clinically well-characterized HD patients during a long-term follow-up, 2) determine which factors (including motor, cognitive and behavioral) are associated with weight loss, 3) assess whether CAG repeat length is directly related to the rate of weight loss, and 4) determine whether CAG repeat length is also associated with body weight and caloric intake in the most widely used transgenic mouse (R6/2) model of HD.

## METHODS

**HD patients.** Participants were from the European Huntington's Disease Initiative (EHDI) study, a randomized placebo-controlled trial over three years to study the effects of riluzole on the progression of HD.<sup>23</sup> For inclusion, participants were required to be between 25 and 65 years of age, to carry a CAG-repeat expansion in the HD gene of  $\geq 36$ , to manifest clinical signs of HD, and yet to be in an early stage of the disease (defined on the Unified Huntington's Disease Rating Scale (UHDRS) as a motor score  $\geq 5$  and Total Functional Capacity (TFC)-score  $\geq 8$ ). Patients on anti-choreatic (neuroleptic) treatment were not included and start of such medication was a predefined end point. In total, 537 HD patients were randomized of whom 379 completed three years of follow-up. One-hundred-fifty-eight (158) HD patients (29.4%) dropped out due to different reasons, e.g. adverse events, suicide and suicide attempts, start of anti-choreatic medication. Here,

we excluded 20 participants (3.7%) from our analyses due to erroneous data on body weight (n=6) or missing data on height (n=14).

**Clinical evaluations.** Demographic data included age, gender and age of onset. Height, weight and the clinical scores on the UHDRS were recorded at baseline. Weight and UHDRS scores were also measured at subsequent visits at 2, 6, 12, 18, 24, 30, 36 and 37 months after the start of the study. Missing values at baseline observations were replaced by the corresponding value obtained at the screening visit wherever available.<sup>23</sup> The UHDRS is divided into four components assessing motor performance, cognition, behavior and functional capacity.<sup>3,24,25</sup> In addition, symptoms of depression were also assessed with the Beck Depression Inventory (BDI).

**R6/2 transgenic mice.** We used eight transgenic HD mice of the R6/2 line and eight wild-type littermates (Jackson Laboratories, Bar Harbor, ME, USA).<sup>13</sup> They were obtained by crossing heterozygous males with females of their background strain (C57BL/6). CAG repeat lengths were assessed using a polymerase chain reaction assay (Mangiarini et al., 1996). The mice were singly housed from five weeks of age and had *ad libitum* access to water and food under standard conditions (12 h light/dark cycle, 22 °C). They were fed a standard diet (15% fat on a caloric basis). As R6/2 mice develop progressive locomotor problems, food was placed in dishes on the bottom of the cage. We monitored food intake four times per week over 24 h by pre-weighing a portion of food and weighing it 24 h later. From week six to week 12, we measured body weight twice per week. At 12 weeks of age, mice were euthanized for ethical reasons. The experimental procedures were approved by the Regional Ethical Committee of Lund University, Sweden.

**Statistical analyses. HD patients:** We used linear mixed-effects models<sup>26</sup> to examine changes in BMI during the follow-up period. To account for the correlation between the repeated measurements on each individual, we used a model with both fixed and random terms for time passed since the start of the trial. Disease duration at the start of the trial was considered as a fixed covariate. We also added the quadratic term for time to investigate potential non-linear relations between BMI and time. However, this term was not significant and was therefore left out. The associations between BMI changes and other demographic and clinical variables such as gender, various UHDRS subscores and CAG repeat length were studied by adding these variables one by one as explanatory variables into the model. To assess whether a variable was significantly associated with the rate of BMI change, also the interaction of this variable with the time variable was added and tested. A significant interaction entails that the variable of concern influences the rate of BMI change. To identify which predictor variables from Table 2 were *independently* associated with the rate of BMI change, we also used a stepwise regression procedure based on forward selection (an independent predictor of BMI change is a variable that remains significantly associated with the rate of BMI change after adjustment for the effects of other significant predictor variables.) Models were validated both graphically and analytically.<sup>26</sup> The linear mixed-effects models procedure applied here is valid under the Missing at Random assumption. Under the Missing at Random assumption drop out of subjects is allowed to depend on both previous outcome measurements as well as predictor variables.<sup>26</sup> In addition, we also verified that drop out of subjects did not depend on any baseline characteristic using a Cox proportional-hazards regression model. Differences between the baseline characteristics of the placebo and the riluzole group were statistically evaluated by the unpaired Student's t-test

or the  $\chi^2$ -test where appropriate. *R6/2 mice*: As R6/2 mice first display reduced growth and later (from nine weeks of age and onwards) lose weight, for each mouse we calculated the total area under the curve for both body weight and caloric intake (kCal per gram of body weight). Pearson's correlation coefficients were then computed to assess correlations.

All data are presented as means  $\pm$  SEM. All tests were two-tailed and values of  $p < 0.05$  were considered to be significant. Programming was performed in SPSS version 14.0 for Windows (SPSS Inc, Chicago, Ill, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

### *HD patients*

#### *Baseline characteristics*

All baseline characteristics of the HD patients who were included are summarized in Table 1. Except for small differences in age at the start of the trial, age of onset and the number of CAG repeats, there were no significant differences between the baseline characteristics of patients on either placebo or riluzole.

#### *BMI and rate of BMI change*

The rate of BMI change seemed to differ significantly between the placebo and the riluzole group (Table 2). However, since the two groups differed on a number of basal characteristics (Table 1), we corrected for these differences by including age, age of onset, CAG repeat number and baseline BMI and their interactions with time in the model. After correction for these confounders the two treatment groups ceased to differ significantly in their rate of BMI change (p-value of group  $\times$  time interaction = 0.126). Moreover, stepwise regression did not identify treatment group as an independent predictor of BMI change (Table 2). Therefore, we based all our subsequent analyses on the pooled data.

At baseline the average BMI of the total study population ( $n = 517$ ) was 23.29 (SEM = 0.16). The mean BMI decreased with -0.15 units per year (SEM = 0.038; 95% CI: -0.23 to -0.08;  $p < 0.001$ ). A higher BMI at baseline was associated with a faster rate of body weight decline (adjusted  $p$  for baseline BMI  $\times$  time interaction = 0.001; Table 2). On average women weighed significantly less than men by about -0.88 BMI units

**Table 1. Baseline characteristics of Huntington's patients that participated in the study**

	<i>Total cohort (n=517)</i>
<b>Group (n)</b>	
<b>Placebo</b>	173 (33.5%)
<b>Riluzole</b>	344 (66.5%)
<b>Men (%)</b>	260 (50.3%)
<b>Age (yrs) <sup>†</sup></b>	45.65 (0.43)
<b>Age of onset (yrs) <sup>†</sup></b>	43.52 (0.44)
<b>Disease duration (yrs)</b>	2.13 (0.09)
<b>BMI (kg/m<sup>2</sup>)</b>	23.29 (0.16)
<b>Years of education</b>	11.33 (0.17)
<b>CAG repeat number <sup>†</sup></b>	45.44 (0.19)
<b>UHDRS motor score</b>	28.21 (0.66)
<b>UHDRS TFC score</b>	10.88 (0.07)
<b>UHDRS FAS score</b>	22.07 (0.13)
<b>UHDRS cognitive score</b>	175.99 (2.79)
<b>UHDRS behavioral score</b>	11.85 (0.43)
<b>BDI score</b>	10.10 (0.39)

Data are presented as means ( $\pm$  SEM). Abbreviations: BMI = Body Mass Index; UHDRS = Unified Huntington's Disease Rating Scale; TFC = Total Functional Capacity; FAS = Functional Assessment; BDI = Beck Depression Inventory; <sup>†</sup> The placebo and the riluzole group differed significantly on these baseline characteristics ( $p < 0.05$ ).

(SEM = 0.322; 95% CI: -1.51 to -0.25;  $p = 0.007$ ). The significant interaction between gender and time ( $p = 0.037$ ; Table 2) disappeared when corrected for BMI at baseline ( $p = 0.078$ ) indicating that men and woman do not differ in their rate of weight loss. Age of disease onset was not associated with BMI or the rate of BMI change (both  $p \geq 0.061$ ; Table 2).

**Table 2. Correlates of BMI and the rate of BMI change in patients with Huntington's disease**

	<i>Predictor variable</i>	<i>Effect on average BMI<sup>a</sup> (p-value)</i>	<i>Effect on rate of BMI change<sup>b</sup> (p-value)</i>	<i>Stepwise forward selection<sup>c</sup> (p-value)</i>
<b>General variables</b>	<i>Age of onset</i>	0.031 (0.061)	0.001 (0.805)	-
	<i>Baseline BMI, kg/m<sup>2</sup></i>	0.997 (<0.001)**	-0.030 (0.005)*	-0.034 (0.001)**
	<i>Gender<sup>d</sup></i>	-0.879 (0.007)**	0.158 (0.037)*	-
	<i>CAG repeat number</i>	-0.136 (<0.001)**	-0.022 (0.017)*	-0.027 (0.006)**
	<i>Group (riluzole/placebo)<sup>e</sup></i>	0.568 (0.098)	-0.202 (0.011)*	-
	<i>Combined score</i>	-0.005 (0.262)	-0.001 (0.698)	-
	<i>UHDRS TFC score</i>	0.012 (0.580)	0.003 (0.827)	-
<b>Motor variables</b>	<i>UHDRS FAS score</i>	0.012 (0.425)	-0.001 (0.949)	-
	<i>UHDRS total motor score</i>	-0.005 (0.172)	-0.002 (0.367)	-
	<i>Chorea</i>	-0.011 (0.242)	-0.005 (0.418)	-
	<i>Dystonia</i>	-0.021 (0.109)	0.005 (0.599)	-
	<i>Rigidity</i>	0.005 (0.853)	-0.030 (0.151)	-
<b>Behavioral variables</b>	<i>Bradykinesia</i>	-0.075 (0.059)	-0.061 (0.038)*	-
	<i>UHDRS total behavioral score</i>	-0.004 (0.292)	-0.002 (0.515)	-
	<i>Total behavioral score frequency</i>	-0.006 (0.330)	-0.004 (0.444)	-
	<i>Total behavioral score severity</i>	-0.009 (0.244)	-0.003 (0.638)	-
	<i>Depression score</i>	-0.020 (0.170)	-0.003 (0.638)	-
	<i>Apathy score</i>	0.003 (0.832)	-0.020 (0.079)	-
	<i>BDI score</i>	-0.008 (0.063)	-0.003 (0.365)	-
<b>Cognitive variables</b>	<i>UHDRS total cognitive score</i>	0.001 (0.291)	<0.001 (0.652)	-
	<i>Verbal Fluency</i>	0.007 (0.089)	0.008 (0.148)	-
	<i>Symbol Digit Test</i>	0.011 (0.028)*	<0.001 (0.956)	-
	<i>Color naming</i>	0.003 (0.430)	0.001 (0.569)	-
	<i>Word reading</i>	-0.001 (0.754)	0.002 (0.136)	-
	<i>Interference</i>	-0.004 (0.355)	-0.001 (0.577)	-

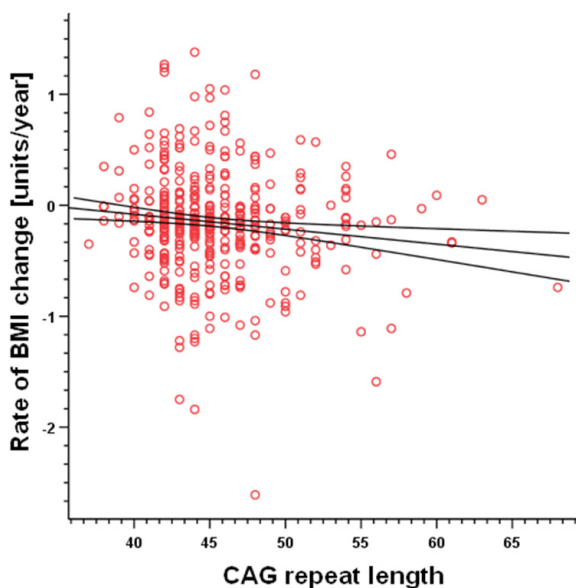
<sup>a</sup>) This column indicates the increase or decrease in average BMI (calculated over the whole study period) per unit increase of the predictor variable. <sup>b</sup>) This column indicates the increase or decrease in the rate of BMI change [BMI units/year] per unit increase of the predictor variable. <sup>c</sup>) This column indicates the increase or decrease in the rate of BMI change [BMI units/year] per unit increase of those predictor variables that were selected by stepwise forward selection; these predictor variables are independently associated with the rate of BMI change. <sup>d</sup>) Gender was coded as: male = 0, female = 1. <sup>e</sup>) Group was coded as: placebo = 0, riluzole = 1. *Abbreviations:* BMI = Body Mass Index; UHDRS = Unified Huntington's Disease Rating Scale; TFC = Total Functional Capacity; FAS = Functional Assessment; BDI = Beck Depression Inventory; \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

Of all the motor signs of HD only bradykinesia was significantly associated with the rate of BMI change (Table 2). Patients who became more bradykinetic during their illness had a significantly accelerated rate of body weight loss compared to others ( $p = 0.038$ ). However, stepwise regression did not identify bradykinesia as an independent predictor of BMI change (Table 2). As the average bradykinesia score increased with 0.023 units for each unit increase in CAG repeat length ( $p = 0.003$ ), CAG repeat length is likely to have confounded the relation between bradykinesia and weight loss.

#### No effect of cognitive and behavioral scores on weight change

Except for the Symbol Digit Modalities test score, which very weakly correlated with BMI, other cognitive and behavioral variables did not correlate with BMI. Moreover, no single cognitive or behavioral variable was associated with the rate of BMI change in the HD cohort (Table 2).

**Figure 1.** Huntington's disease patients with larger CAG repeat lengths have a faster rate of weight loss. The straight line represents the regression line, while the outer lines delineate the 95% confidence intervals.



#### Lower mean BMI and faster rate of BMI decline with larger CAG repeat number

Interestingly, the BMI averaged over the follow-up period decreased with 0.136 units for every CAG codon increase in the mutant *huntingtin* gene ( $p < 0.001$ ). Moreover, the number of CAG repeats in the mutant *huntingtin* gene significantly interacted with the time variable ( $p = 0.017$ ), indicating that the rate of body weight decline also increases for each unit increase in CAG repeat length (Table 2 and Figure 1). Stepwise regression identified CAG repeat length also as an independent predictor of weight loss (adjusted  $p$  for CAG  $\times$  time interaction = 0.006).

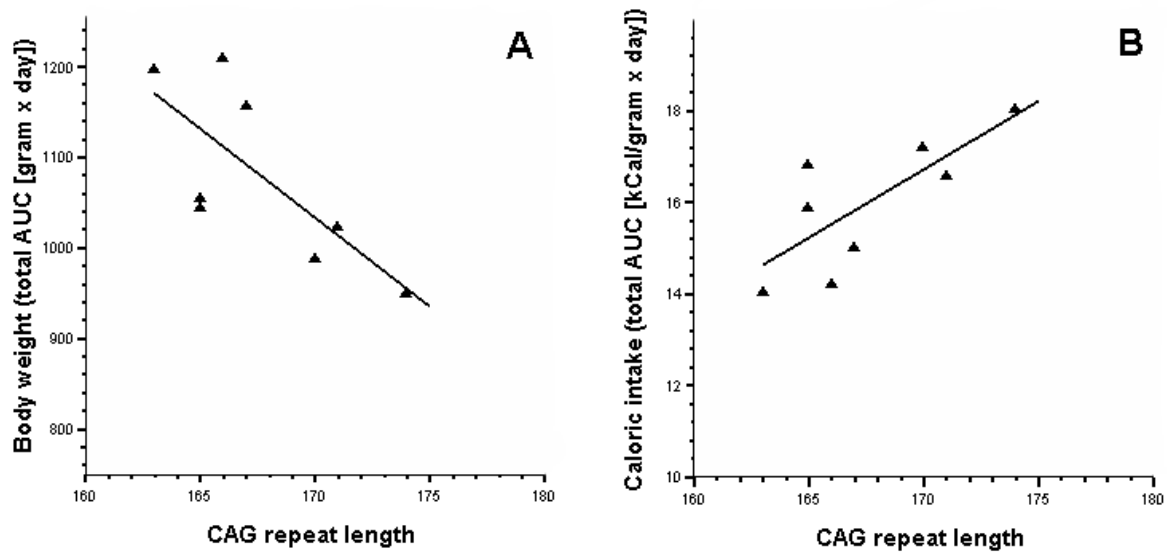
#### R6/2 transgenic mice

Similar to their wild-type littermates, R6/2 mice gained weight from the start of the study at six weeks of age until week nine.<sup>13</sup> However, from nine

weeks of age and onwards, they progressively lost weight. The length of the CAG repeat in the transgene in our cohort of R6/2 mice varied between 163 and 175. The area under the body weight curve decreased with larger CAG repeat length ( $r = -0.742$ ;  $p = 0.035$ ), indicating that mice with a higher number of CAG repeats have a lower body weight. Conversely, the area under the caloric intake curve increased with larger CAG repeat length ( $r = 0.763$ ;  $p = 0.028$ ); i.e. R6/2 mice with higher repeat lengths consume more energy per gram of body weight.



**Figure 2.** In R6/2 mice, a greater number of CAG repeats correlates with lower body weight ( $r = -0.742$ ;  $p = 0.035$ ) (A), and higher caloric intake ( $r = 0.763$ ;  $p = 0.028$ ) (B). AUC = area under the curve.



## DISCUSSION

We conducted a long-term follow-up study of body weight changes in a large group of HD patients who were at an early stage of the disease and not on neuroleptic treatment. We found a significant decrease in body weight; however, no single motor, cognitive or behavioral score was independently associated with weight loss. As weight loss in R6/2 mice is also not related to motor activity<sup>13</sup>, our findings suggest that loss of body weight in HD is not secondary to hyperactivity or other symptoms, but rather results from a hypermetabolic state. As both HD patients and transgenic mice showed a higher rate of weight loss with greater CAG repeat number, this hypermetabolic state is likely to stem directly from interference of the mutant protein with cellular energy homeostasis. Weight loss could therefore reflect fundamental pathological mechanisms underlying HD and may serve as a biomarker to monitor disease progression. Moreover, patients with a higher number of CAG repeats are at increased risk of unintended weight loss. Therefore, their body weight should be monitored more closely.

Our findings indicate that weight loss is an inherent feature of HD and are in agreement with many other observations.<sup>3,4,6,14</sup> Indices of increased motor activity, such as chorea and dystonia, did not correlate with the rate of weight loss, neither did the total motor score of the UHDRS. Weight loss is therefore unlikely to result from hyperactivity. Although weight loss correlated with bradykinesia, this relation is likely confounded by CAG repeat number as bradykinesia increased with higher CAG repeat number and did not correlate with weight loss when adjusted for baseline BMI and CAG repeat number. Furthermore, cognitive impairment, as measured by the Symbol Digit Modalities test, weakly correlated with mean body weight. Cognitive impairment might cause more disability.<sup>20</sup> However, total functional capacity and ratings on the independence scale, both measures of disability, were not associated with weight loss. Therefore, it seems unlikely that there is a causative link between specifically bradykinesia or cognitive impairment and body weight loss. Although

we did not collect data regarding caloric intake, a decrease in energy intake is also unlikely to account for the weight loss. This is because all patients were at an early stage of the disease which is generally associated with increased rather than decreased caloric intake.<sup>6,11,14</sup> Similarly, R6/2 mice also do not exhibit decreased caloric intake until two weeks after the commencement of weight loss, indicating that decreased caloric intake is not the cause of weight loss.<sup>13</sup>

Here we demonstrate that CAG repeat number in mutant *huntingtin* is directly related to both the average body weight during follow-up and the rate of weight decline in HD patients. This extends upon earlier studies that have found associations between CAG repeat length and several other clinical features of HD, particularly age of disease onset.<sup>27</sup> Moreover, CAG repeat length has also been shown to correlate with the rate of disease progression as assessed by the extent of post mortem<sup>28,29</sup> or *in vivo*<sup>30-32</sup> striatal pathology. Interestingly, R6/2 mice with larger CAG repeat lengths had also lower body weights, despite relatively small differences in CAG repeat number between individual mice. When comparing different HD transgenic mouse models, CAG repeat length correlates with a number of biochemical abnormalities, such as decreases in brain N-acetyl aspartate levels.<sup>33</sup> However, the effect of small variations in CAG repeat number within the same mouse model is not known. Our findings suggest that even relatively small differences (up to 12) in the number of CAG repeats within the same transgenic strain may lead to phenotypic dissimilarities in, e.g., body weight.

Several mechanisms could account for the negative association between CAG repeat length and body weight. Mitochondrial dysfunction has long been implicated in HD pathogenesis as markers of energy metabolism are altered in HD brain, muscle<sup>34,35</sup> and lymphoblastoid cells<sup>21</sup>. The extent of mitochondrial dysfunction may critically depend on the length of the polyglutamine tract, as CAG repeat size has been shown to affect both mitochondrial depolarization and ATP/ADP ratio in lymphoblastoid cells.<sup>21,22</sup> Consequently, longer CAG expansions may cause both more central and peripheral pathology. Larger CAG repeat size has indeed been associated with more severe pathology in the striatum and cortex<sup>28,29,36</sup> and might also be related to more pathology in other brain structures, such as the hypothalamus, which are directly involved in energy homeostasis.<sup>10,37</sup> Interestingly, hypothalamic pathology occurs in both HD patients and transgenic mice.<sup>10,13,38</sup> Longer CAG repeats may cause more extensive changes in peripheral tissues as well. A recent study found reduced levels of branched chain amino acids in HD patients the levels of which were lower with increasing CAG repeat number.<sup>14</sup> Importantly, the levels of these amino acids were also associated with weight loss.<sup>14</sup> Similarly, we found that R6/2 mice with longer CAG repeats had lower body weights, whereas caloric intake was higher in mice with longer repeat lengths. Finally, mutant huntingtin with longer polyglutamine stretches might interfere more strongly with the function of the wild-type protein<sup>39</sup>, which has been shown to influence body weight in some transgenic HD mice.<sup>40</sup>

Only two prior studies have investigated body weight changes in large groups of HD patients.<sup>19,20</sup> As weight loss is commonly considered a feature of HD<sup>10</sup>, it is surprising that weight loss was not observed in these cohorts. In all likelihood, the heterogeneity of these cohorts combined with lack of clinical information on e.g. the use of nutritional supplements and drugs (notably neuroleptics) could account for the unexpected findings.<sup>19,20</sup> Moreover, CAG repeat length data were not available for these studies. In contrast, our cohort

of HD patients was very homogenous, consisting of patients at an early stage of the disease. Importantly, participants were also required not to be on neuroleptic treatment during the trial. Since neuroleptics are applied frequently in HD and often substantially influence systemic energy homeostasis, the EHDI cohort is the first large group of HD patients in which body weight changes could be investigated without confounding neuroleptic medication. Although our data were derived from a clinical trial with riluzole, riluzole treatment was reported not to affect any clinical outcome measure in this cohort<sup>23</sup>. We confirmed this and also showed that riluzole was not an independent predictor of weight change. Therefore, it is highly unlikely that riluzole treatment might have influenced our findings.

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### Normal and mutant *HTT* interact to affect clinical severity and progression in Huntington's disease

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## ABSTRACT

*Objective:* Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the HD gene (*HTT*). We aimed to assess whether interaction between CAG repeat sizes in the mutant and normal allele could affect disease severity and progression. *Methods:* Using linear regression and mixed-effects models, the influence of mutant and normal CAG repeat sizes interaction was assessed on: (1) age of onset in 921 HD patients, (2) clinical severity and progression in 512 of these patients with follow-up data available, and (3) basal ganglia volume on magnetic resonance images in 16 premanifest HD mutation carriers. *Results:* Normal and mutant CAG repeat sizes interacted to influence: (1) age of onset ( $p=0.001$ ), (2) severity or progression of motor, cognitive and functional, but not behavioral, symptoms in HD patients (all  $p<0.05$ ), and (3) in premanifest subjects, basal ganglia volumes ( $p<0.05$ ). In subjects with mutant CAG expansions in the low range, increasing size of the normal repeat correlated with more severe symptoms and pathology, whereas for those subjects with expansions in the high range, increasing size of the normal repeat correlated with less severe symptoms and pathology. *Conclusions:* Increasing CAG repeat size in normal *HTT* diminishes the association between mutant CAG repeat size and disease severity and progression in HD. The underlying mechanism may involve interaction of the polyglutamine domains of normal and mutant huntingtin (fragments) and needs further elucidation. These findings may have predictive value and are essential for the design and interpretation of future therapeutic trials.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the *HTT* gene, resulting in a long polyglutamine tract in the N-terminus of the encoded protein huntingtin.<sup>1</sup> The disease is characterized by motor impairment, cognitive deterioration and behavioral disturbances.<sup>2</sup> The size of the CAG repeat sequence in the mutant allele is inversely related to age of onset, accounting for up to 73% of the variance.<sup>3</sup> Moreover, larger mutant CAG repeat sizes correlate with an increased rate of deterioration on motor, cognitive and functional domains as well as weight loss, whereas behavioral symptoms appear not to be related to repeat size.<sup>4-6</sup>

It has been suggested that the polyglutamine stretch in normal huntingtin could bind to the expanded polyglutamine stretch, and thereby modulate the effects of mutant huntingtin or result in loss of function of the normal protein.<sup>7-9</sup> Indeed, interaction between CAG repeat sizes in the mutant and normal allele is reported to influence age of onset, with larger sizes of the normal repeat being associated with a delayed age of onset in persons having large mutant CAG repeat sizes.<sup>10-13</sup> However, it is not known whether interaction between mutant and normal huntingtin could also influence clinical signs and progression of the disease in HD patients, or affect indices of immanent phenocconversion in premanifest HD mutation carriers.

Therefore, this study was undertaken to test the hypothesis that CAG repeat sizes in the mutant and normal allele interact to influence not only age of onset, but also clinical severity and progression in HD patients, as well as brain pathology in premanifest HD mutation carriers.

## METHODS

### *HD patients*

We used monitored data from the European Huntington Disease Network (EHDN) Registry study collected prior to December 31<sup>st</sup> 2007. Registry – a multi-centre, prospective, observational study – is EHDN's core study. It was established in 2004 to collect phenotypical data and biomaterials from a large group of HD patients. The study aims to enlist one third of the European HD population by 2010. More information about the Registry cohort can be found at '<http://www.euro-hd.net/html/registry>'. In order to assess interactive effects of mutant and normal CAG repeat sizes on age of onset, we included all Registry participants with a clinical diagnosis of HD whose ages of onset and CAG repeat numbers in both alleles (mutant allele  $\geq 36$  repeats) were available (n=921). To assess the effects on clinical severity and disease progression, only those patients with two or more measurement occasions were included (n=512). The clinical characteristics of all HD patients are summarized in Table 1.

### *Clinical evaluations*

Demographic data included age, gender and age of onset. Age of onset was defined as the age at which, according to the rater, the first motor, cognitive or behavioral signs of HD appeared. Visit data were collected annually (+/- 3 months). Clinical severity was assessed using the Unified Huntington Disease Rating Scale (UHDRS), and was recorded at all visits.<sup>14-16</sup> In addition, body weight was recorded at every visit.

### ***Premanifest HD mutation carriers***

Sixteen premanifest subjects were included whose characteristics are summarized in Table 1. Premanifest status was defined as the absence of unequivocal HD signs by a neurologist specialized in movement disorders (R.A.C.R.).<sup>17</sup> In all premanifest subjects, magnetic resonance imaging (MRI) was performed to assess caudate, putamen and globus pallidus volumes, using a 3.0 Tesla scanner (Philips Medical Systems, Best, The Netherlands) as described previously.<sup>17</sup>

### ***Standard Protocol Approvals, Registrations, and Patient Consents***

Full ethical approval has been obtained for each European country contributing to the Registry study. The MRI study was approved by the medical ethics committee of the Leiden University Medical Center. All subjects gave written informed consent.

**Table 1. Characteristics of the study populations**

	<b>Group I<sup>† a</sup></b>	<b>Group II<sup>† b</sup></b>	<b>Group III<sup>† c</sup></b>
<i>No. of subjects (n)</i>	921	512	16
<i>Male (%)</i>	47.9	47.5	37.5
<i>Age of onset, y</i>	42.9 (12.0; 6–79)	42.9 (11.8; 9–79)	-
<i>Age at MRI, y</i>	-	-	42.4 (10.1; 24–59)
<i>Disease duration, y*</i>	7.1 (5.3; 0–40.3)	7.0 (4.9; 0–29.5)	-
<i>UHDRS total motor score*</i>	35.3 (21.4; 0–106)	34.7 (20.6; 0–105)	-
<i>UHDRS TFC score*</i>	8.2 (3.7; 0–13)	8.2 (3.6; 0–13)	-
<i>Mutant CAG repeat size</i>	44.9 (5.0; 36–90)	44.9 (4.5; 36–90)	42.4 (2.7; 40–49)
<i>Normal CAG repeat size</i>	18.7 (3.2; 9–37) <sup>‡</sup>	18.6 (3.1; 9–32)	17.8 (3.2; 9–23)

\*Disease duration, total motor score and total functional capacity (TFC) score are indicated at the first study visit.

<sup>†</sup> All data are indicated as means (standard deviation; range).

<sup>‡</sup> There was one homozygous subject in this cohort with 37 and 42 repeats; exclusion of this subject did not change the results.

<sup>a)</sup> The total Registry cohort with data available on age of onset and CAG repeat sizes. <sup>b)</sup> All the patients from the total Registry cohort with two or more follow-up measurements; patients with and without follow-up data did not differ with respect to age, sex, body mass index, age of onset, mutant and normal CAG repeat size, and disease duration, total motor score and total functional capacity at first visit (all  $p \geq 0.223$ ; unpaired t-tests and  $\chi^2$ -test). <sup>c)</sup> Premanifest HD gene carriers. MRI = Magnetic Resonance Imaging, TFC = Total Functional Capacity, UHDRS = Unified Huntington Disease Rating Scale.

## Statistical analyses

To assess whether age of onset is influenced by the interaction between mutant and normal CAG repeat sizes, we used multiple linear regression. As the relation between age of onset and mutant CAG repeat size is known to be exponential<sup>3</sup>, we used the logarithmic transform of age of onset as the dependent variable. The sizes of the mutant and normal CAG repeats, and their interaction, were used as predictor variables. To examine whether clinical scores during the follow-up period were influenced by the interaction between mutant and normal CAG repeat number, we used linear mixed-effects models<sup>18</sup> with the clinical scores as dependent variables. We used random effects to account for the correlation between the repeated measurements on each individual. In all models, both random intercepts and slopes were used for disease duration to reflect the heterogeneity in terms of baseline levels and evolution with time. Age of onset was always included as a covariate, since it can confound the relation between clinical phenotype and CAG repeat number.<sup>4</sup> Other explanatory variables included mutant CAG repeat number and its interaction with disease duration, normal CAG repeat number and its interaction with disease duration, an interaction term between mutant and normal CAG repeat sizes, and an interaction term between disease duration and mutant and normal CAG repeat sizes. Thus, an interaction term in which disease duration is not included assesses whether two variables interact on the dependent variable at the mean time of follow-up, whereas an interaction term in which disease duration is included assesses whether an independent variable (or the combination of two independent variables in case of a three-way interaction) influences the *rate* of progression. In order to visualize the results, we drew regression lines based on the predictions of the mixed-effects models for different groupings of mutant and normal CAG repeat sizes, representing the association between clinical features and disease duration. In premanifest subjects, multiple linear regression was applied to assess whether CAG repeat sizes interact to affect basal ganglia volumes. Volumes of the caudate, putamen, globus pallidus, and their sum were used as dependent variables, while the sizes of the mutant and normal alleles, and their interaction, were used as predictor variables. In order to reduce multicollinearity, particularly with respect to the interaction terms, and simplify the interpretation of results, disease duration, age of onset, and mutant and normal CAG repeat sizes were centered around their respective means. We did not apply a specific correction for multiple testing because (1) based on previous literature we could formulate an a priori hypothesis presuming a relation between CAG repeat sizes interaction and disease severity and progression, and (2) there is a high degree of interdependence between various measures of clinical severity, i.e. the UHDRS scores cannot be regarded as independent of one another. Therefore, all tests were two-tailed and values of  $p < 0.05$  were considered to be significant. Programming was performed in SPSS version 14.0 for Windows (SPSS Inc, Chicago, Ill, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

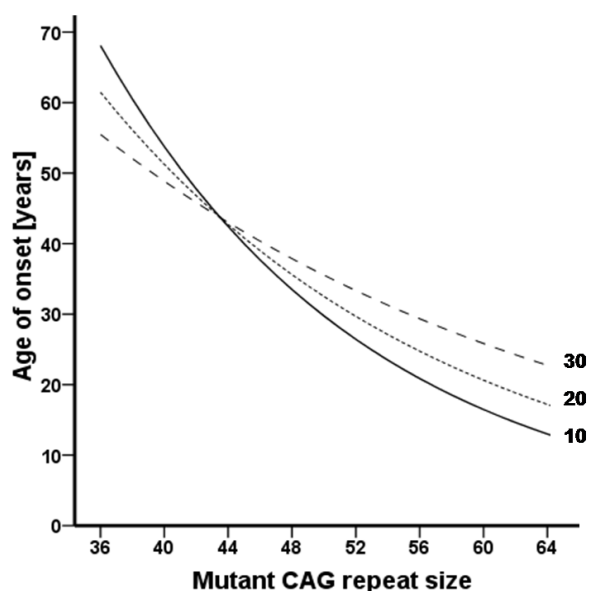
### *Age of onset*

The clinical characteristics of this cohort of HD patients (group I) are summarized in the second column of table 1. The interaction term between mutant and normal CAG repeat sizes was highly significant (table E-1). The inclusion of the interaction term raised the adjusted  $R^2$  from 0.529 to 0.534, indicating that the model with the interaction term included, can account for 53.4% of the variance in the age of onset. Model predicted best fit lines (figure 1), revealed that in the low range of mutant CAG repeat size, higher normal CAG repeat sizes



are related to a lower age of onset, while in the high range of the mutant repeat size, higher values of the normal repeat size are related to a higher age of onset. Thus, the association between mutant CAG repeat size and age of onset progressively weakens for higher normal CAG repeat sizes (figure 1).

**Figure 1. Normal and mutant CAG repeat sizes interact to affect age of onset in HD patients.** The relation between age of onset and mutant CAG repeat size progressively weakens with increasing normal CAG repeat size. Solid line, line with small dashes, and line with large dashes represent the relation between CAG repeat size in the mutant allele and age of onset for, successively, 10, 20 and 30 CAG repeats in the normal allele.



### Clinical severity and progression

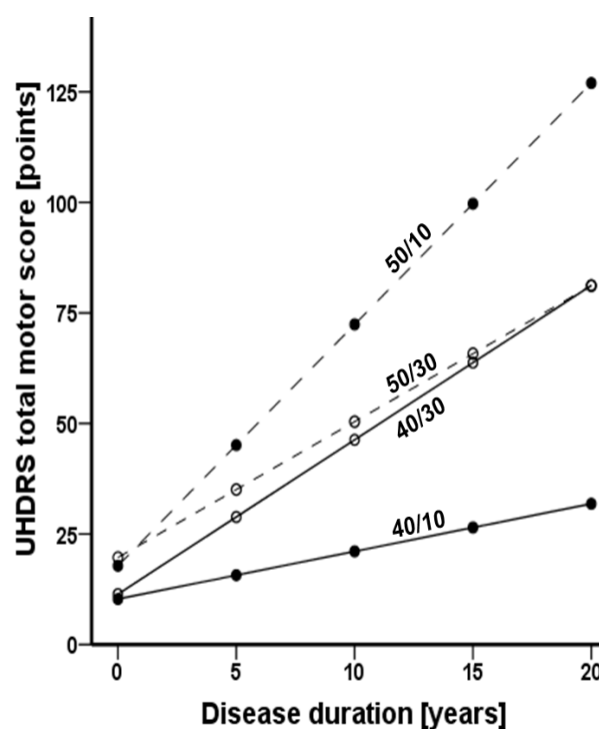
The clinical characteristics of the HD patients of whom follow-up data were available (group II) are summarized in the third column of table 1. Interaction between mutant and normal CAG repeat sizes significantly influenced the mean — at average disease duration — of the UHDRS total motor score, total cognitive score, and total functional capacity (TFC), but not the mean total behavioral score or body mass index (table E-2). The regression coefficients in table E-2 indicate that increasing normal CAG repeat size was associated with more severe symptoms in the low range of the mutant CAG repeat size, but with less severe symptoms in the high range of the mutant CAG repeat size. For example, in case of 40 CAG repeats in the mutant allele, for each unit increase in the normal CAG repeat size the mean motor score increased with 0.80 points, the mean cognitive score decreased with 3.05 points, and the mean TFC score decreased with 0.10 points. However, in case of 50 CAG repeats in the mutant allele, for each unit increase in the normal CAG repeat size the mean motor score decreased with 0.74 points, the mean cognitive score increased

with 2.16 points, and the mean TFC score increased with 0.08 points. In addition, the rate of progression of the UHDRS total motor score was influenced by the interaction between the two CAG repeat sizes (table E-2). Model predictions of total motor score during follow-up revealed that, in the lower range of the mutant CAG repeat sizes, increasing normal CAG repeat size was associated with a faster rate of motor progression, while in the upper range of mutant CAG repeat size, increasing normal CAG repeat size was associated with a slower rate of motor progression (figure 2, appendix E-1). Similar plots were generated for the total cognitive score, total behavioral score, total functional capacity, and body mass index. Except for the total behavioral score, these graphs showed a similar pattern as the total motor score, although the differences were generally less pronounced (data not shown).



**Figure 2. Normal and mutant CAG repeat sizes interact to affect disease progression in HD patients.**

The relation between motor progression and mutant CAG repeat size is different for various values of normal CAG repeat size. The graph shows model predicted best fit lines based on the median age of onset of 43 years for four different combinations of mutant and normal CAG repeat sizes: 40/10 (solid line and filled circles), 40/30 (solid line and open circles), 50/10 (dashed line and filled circles), and 50/30 (dashed line and open circles). Note that for large normal CAG repeat sizes — e.g. 30 in this case — the effect of the mutant CAG repeat size on disease progression is diminished, i.e. disease progression rate is very similar for 40/30 and 50/30 CAG repeat combinations. UHDRS = Unified Huntington Disease Rating Scale.



### Basal ganglia volumes in premanifest subjects

The clinical features of this cohort of premanifest HD gene carriers (group III) are displayed in the last column of table 1. Volumes of the basal ganglia as a whole, as well as the putamen alone, were significantly affected by the interaction between the mutant and normal CAG repeat sizes, while the interaction effect was borderline significant in case of the caudate nucleus ( $p = 0.091$ ) and globus pallidus ( $p = 0.057$ ) volumes (table E-3). Again, when accounting for the centering of the data, the direction of the interaction effect was opposite to that of the effect of the mutant CAG repeat size. This indicates that, for a given mutant repeat size, larger sizes of the normal repeat weaken the association between mutant repeat size and basal ganglia volume.

## DISCUSSION

We found that normal CAG repeat size interacts with mutant CAG repeat size to affect both clinical severity and progression in HD patients, as well as brain atrophy in premanifest HD mutation carriers. These findings represent a major extension upon previous reports on CAG repeat sizes interaction, all of which used age of onset as the sole outcome measure.<sup>10,11,13</sup> Here we demonstrate that, in addition to age of onset, many signs and symptoms of HD, including motor, cognitive and functional indices, are also influenced by the interaction between CAG repeat sizes in the normal and mutant allele. As the effect of the interaction on basal ganglia volume could already be detected in premanifest subjects, our data suggest that the interplay between normal and mutant huntingtin (fragments) directly influences neuronal atrophy or loss and is thus an inherent feature of HD pathogenesis.

Several models could account for our findings, including competitive polyglutamine length dependent interaction of normal and mutant huntingtin with numerous protein binding partners,<sup>19,20</sup> mitochondrial energy production<sup>21</sup> or transcriptional mechanisms.<sup>22</sup> However, as mutant N-terminal huntingtin fragments can promote the fibrillogenesis and co-aggregation of normal huntingtin fragments, with an increasing rate for larger polyglutamine stretches in either the normal or the mutant range,<sup>23,24</sup> the simplest model would be to assume that the rate of co-aggregate formation is proportional to the polyglutamine stretch in either the normal or the mutant protein.<sup>23,24</sup> This could explain the observation that the association between mutant CAG repeat size and clinical and pathological severity was weakened with larger normal CAG repeat sizes, since increasing stretches of polyglutamine in normal huntingtin could lead to a stronger association with mutant protein fragments, promoting their co-aggregation and preventing them from aberrantly interfering with other proteins.<sup>20,25</sup> Indeed, a number of studies have found that normal huntingtin can reduce the cellular toxicity of the mutant protein both *in vitro* and *in vivo*.<sup>7,26,27</sup> However, a stronger interaction between normal and mutant huntingtin could also result in a greater degree of loss of normal huntingtin function.<sup>23</sup> There is now considerable evidence indicating that normal huntingtin is essential for neuronal function and survival, that it can protect cells from a host of toxic stimuli, and that loss of normal huntingtin function is likely to be involved in HD pathogenesis.<sup>8,9,27-30</sup> Therefore, the interaction between mutant and normal huntingtin could have both beneficial (i.e. mitigation of mutant protein toxicity) and detrimental (i.e. loss of normal huntingtin function) effects.<sup>23,25</sup> The finding that in subjects with mutant CAG expansions in the low range, increasing size of the normal repeat correlated with more severe symptoms and pathology, suggests that in the low range of mutant polyglutamine stretches, the net effect of this interaction is likely to be detrimental due to loss of normal huntingtin function. Conversely, as in subjects with expansions in the high range increasing size of the normal repeat correlated with less severe symptoms and pathology, the net effect of this interaction in the high range of the mutant polyglutamine sequences is likely to be beneficial due to mitigation of mutant protein toxicity.

As we show that the normal *HTT* allele can also influence disease severity, our findings are in line with recent studies in transgenic mouse models of HD<sup>31</sup> and indicate that HD displays an intermediate dominant phenotype in humans as well. These findings challenge the classical common view of HD as a disorder with complete dominance, which is largely based on the clinical evaluation of small groups of potential homozygotes prior to the discovery of the genetic mutation.<sup>32,33</sup> More recent clinical, imaging and neuropathological assessment of genetically confirmed homozygote patients with HD, however, did indeed reveal an increased rate of disease progression.<sup>34</sup> Additionally, our findings are supported by recent studies in transgenic HD mice with super-long CAG repeat expansions which show that homozygous transgenic mice with CAG repeat expansions of around 400 in both transgenes do not exhibit a noteworthy acceleration of phenotype compared to mice with only one 400 CAG transgene, whereas homozygous mice with one transgene in the 350 CAG range and one in the 400 CAG range, do show an accelerated phenotype.<sup>35,36</sup> Together these findings underscore that the exact as well as the relative sizes of the CAG repeat tract in both *HTT* alleles are important determinants of pathology in HD.

Potential limitations of our study include the lack of follow-up data on a subset of HD patients and the relatively short follow-up period. However, patients with and without longitudinal data did not differ in any particular

way (table 1), indicating that lack of follow-up in some cases is unlikely to have biased the results. As the Registry cohort continues to accrue, more precise estimates of the clinical effects of the CAG repeat sizes interaction will be possible in the future.

## ACKNOWLEDGEMENTS

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**Table E-1. Effect of interaction between mutant and normal CAG repeat sizes on natural log-transform of age of onset in Huntington disease patients.**

	Regression coefficients <sup>†</sup> (95% CI)	p-values
<i>Mean value</i>	3.711 (3.697, 3.725)	<0.001
<i>mCAG<sup>‡</sup> effect</i>	-0.047 ( -0.050, -0.044)	<0.001
<i>nCAG<sup>‡</sup> effect</i>	0.002 (-0.002, 0.006)	0.394
<i>mCAG × nCAG<sup>‡</sup> interaction effect</i>	0.001 (0.001, 0.002)	0.001

<sup>†</sup>) All values and effect sizes are centered around the mean mutant CAG repeat size of 44.901, and mean normal CAG repeat size of 18.673. CI = Confidence Interval.

<sup>‡</sup>) mCAG = mutant CAG repeat size; nCAG = normal CAG repeat size.



**Table E-2. Mutant and normal CAG repeat sizes interact to influence clinical severity and progression in Huntington disease patients**

	Mean value (SE) <sup>a</sup>	Age of onset effect (SE) <sup>a, b</sup> points/y	Time effect (SE) <sup>a, c</sup> points/y	mCAG effect (SE) <sup>a, d</sup> points/ repeat	mCAG × time effect (SE) <sup>a, e</sup> points/ repeat/y	nCAG effect (SE) <sup>a, f</sup> points/ repeat	nCAG × time effect (SE) <sup>a, g</sup> points/ repeat/y	mCAG × nCAG effect (SE) <sup>a, h</sup> points/repeat/ repeat	mCAG × nCAG × time effect (SE) <sup>a, i</sup> points/repeat/ repeat/y
<i>Total motor score</i>	37.182 (0.772)	0.620 (0.083)***	3.242 (0.157)***	2.401 (0.236)***	0.231 (0.042)***	0.102 (0.256)	0.004 (0.050)	-0.164 (0.046)***	-0.024 (0.012)*
<i>Total cognitive score</i>	159.472 (3.570)	-3.132 (0.397)***	-8.270 (0.755)***	-7.645 (1.056)***	-0.553 (0.194)**	-0.517 (1.163)	-0.189 (0.233)	0.521 (0.191)**	0.035 (0.055)
<i>Total behavioral score</i>	11.418 (0.517)	-0.062 (0.060)	0.035 (0.119)	-0.116 (0.173)	-0.037 (0.032)	0.117 (0.163)	0.002 (0.037)	0.003 (0.031)	-0.009 (0.009)
<i>TFC score</i>	7.792 (0.137)	-0.108 (0.016)***	-0.559 (0.028)***	-0.314 (0.043)***	-0.034 (0.007)***	-0.009 (0.045)	-0.003 (0.009)	0.018 (0.008)*	0.001 (0.002)
<i>BMI</i>	23.817 (0.164)	0.027 (0.619)	-0.017 (0.033)	-0.136 (0.051)**	-0.008 (0.008)	0.009 (0.053)	0.001 (0.010)	0.016 (0.010)	0.002 (0.003)

<sup>a</sup>) All values and effect sizes are centered around the mean age of onset of 42.853 years, mean disease duration of 6.996 years, mean mutant CAG repeat size of 44.861, and mean normal CAG repeat size of 18.633. BMI = Body Mass Index; mCAG = mutant CAG repeat size; nCAG = normal CAG repeat size; SE = Standard Error, Time = disease duration; TFC = Total Functional Capacity; y = year. \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

<sup>b</sup>) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per year increase of age of onset (at mean values of disease duration, and mutant and normal CAG repeat sizes).

<sup>c</sup>) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per year increase of disease duration (at mean values of age of onset, and mutant and normal CAG repeat sizes).

<sup>d</sup>) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of mutant CAG repeat size (at mean values of age of onset, disease duration and normal CAG repeat size).

<sup>e</sup>) This column indicates the increase or decrease in the rate of change (points/year) of the Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of mutant CAG repeat size (at mean values of age of onset and normal CAG repeat size).

<sup>f</sup>) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of normal CAG repeat size (at mean values of age of onset, disease duration and mutant CAG repeat size).

<sup>g</sup>) This column indicates the increase or decrease in the rate of change (points/year) of the Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of normal CAG repeat size (at mean values of age of onset and mutant CAG repeat size).

<sup>h</sup>) This column indicates the effect of the mutant and normal CAG repeat sizes interaction on the average Unified Huntington Disease Rating Scale total scores and body mass index (at mean values of age of onset and disease duration).

<sup>i</sup>) This column indicates the effect of the mutant and normal CAG repeat sizes interaction on the rate of change of the Unified Huntington Disease Rating Scale total scores and body mass index (at mean value of age of onset).



Table E-3. Effects of interaction between mutant and normal CAG repeat sizes on basal ganglia volume in premanifest subjects

	Mean value <sup>†</sup> (SE)	Age effect <sup>†</sup> (SE)	mCAG effect <sup>†</sup> (SE)	nCAG effect <sup>†</sup> (SE)	mCAG × nCAG interaction effect <sup>†</sup> (SE)
<i>Caudate volume</i>	6.757 (0.296)	-0.066 (0.025)*	-0.016 (0.098)	-0.307 (0.111)*	-0.111 (0.060)
<i>Putamen volume</i>	6.527 (0.286)	-0.069 (0.024)*	-0.091 (0.095)	-0.268 (0.108)*	-0.161 (0.058)*
<i>Globus pallidus volume</i>	1.434 (0.137)	-0.035 (0.011)*	-0.066 (0.045)	-0.106 (0.052)	-0.059 (0.028)
<i>Total basal ganglia volume</i>	14.718 (0.600)	-0.170 (0.050)**	-0.172 (0.199)	-0.681 (0.226)*	-0.331 (0.121)*

<sup>†</sup>) All values and effect sizes are centered around the mean age of 42.445, mean mutant CAG repeat size of 42.438, and mean normal CAG repeat size of 17.813.

\* p < 0.05; \*\* p < 0.01

## Appendix E-1. Estimating disease progression

The coefficients provided in table E-2 can be used to estimate the clinical scores of HD patients while accounting for age of onset, as well as mutant and normal *HTT* CAG repeat sizes. For example, the following equation can be used to estimate the UHDRS total motor score:

$$\begin{aligned} \text{Total motor score} \approx & 37.182 + 0.620 \times (\text{age of onset} - 42.853) + 3.242 \times (\text{disease duration} - 6.996) + 2.401 \times (\text{mutant} \\ & \text{CAG repeat size} - 44.861) + 0.231 \times (\text{mutant CAG repeat size} - 44.861)(\text{disease duration} - 6.996) + 0.102 \times (\text{normal} \\ & \text{CAG repeat size} - 18.633) + 0.004 \times (\text{normal CAG repeat size} - 18.633)(\text{disease duration} - 6.996) - 0.164 \times (\text{mutant} \\ & \text{CAG repeat size} - 44.861)(\text{normal CAG repeat size} - 18.633) - 0.024 \times (\text{mutant CAG repeat size} - 44.861)(\text{normal} \\ & \text{CAG repeat size} - 18.633)(\text{disease duration} - 6.996) \end{aligned}$$

The effect of the interaction between mutant and normal CAG repeat size on the total motor score can become substantial over time. This can be illustrated by using the above equation. Suppose individuals A and B both have an age of onset of 43 years and an expansion of 40 CAG repeats in their mutant alleles, while the number of repeats in their normal alleles is different: 10 for person A and 30 for person B. Five years after disease onset, the UHDRS total motor score of person A will equal 16 points ( $\approx 37.182 + 0.620 \times (43 - 42.853) + 3.242 \times (5 - 6.996) + 2.401 \times (40 - 44.861) + 0.231 \times (40 - 44.861)(5 - 6.996) + 0.102 \times (10 - 18.633) + 0.004 \times (10 - 18.633)(5 - 6.996) - 0.164 \times (40 - 44.861)(10 - 18.633) - 0.024 \times (40 - 44.861)(10 - 18.633)(5 - 6.996)$ ), whereas person B will have a score of 29 points. A considerable difference of 81% after only five years, that, in addition, will continue to increase over time (see also figure 2 in the manuscript).



### **Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease**

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***Parkinsonism Rel Disorders (accepted for publication)***

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## ABSTRACT

*Objective:* Sleep disturbances are a prominent feature of Huntington's disease (HD) and can substantially impair patients' quality of life. However, sleep complaints and their association with other symptoms and signs of HD have not yet been assessed in large groups of patients or premanifest mutation carriers. Therefore, we aimed to (1) delineate the nature of subjective sleep disturbances, (2) identify important predictors of sleep impairment, and 3) evaluate the usefulness of the SCOPA-SLEEP questionnaire, a short instrument that can assess both daytime sleepiness (DS) and night-time sleep (NS), in a group of HD patients and premanifest mutation carriers. *Subjects & methods:* Using standardized questionnaires, DS, NS and depressed mood were assessed in 63 HD patients, 21 premanifest mutation carriers and 84 controls. *Results:* NS impairment was significantly more prevalent in HD patients compared with controls (58.1% vs. 34.9%,  $p=0.012$ ), but DS was not (12.7% vs. 7.9%,  $p=0.560$ ). Depression was the only independent predictor of NS impairment in HD patients, accounting for 19% of the variance. Compared with controls, both sleep onset latency and wake-up time were significantly delayed in HD patients. Moreover, in HD patients, later wake-up time was significantly associated with cognitive score ( $r=-0.43$ ), total functional capacity ( $r=-0.54$ ) and depressive symptoms ( $r=+0.47$ ). The SCOPA-SLEEP questionnaire was a reliable and valid instrument for application in HD patients. *Conclusions:* HD is primarily accompanied by NS disturbances and a delayed sleep phase syndrome-like phenotype, which are associated with depression and lower cognitive as well as functional performance.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat sequence in the gene encoding the protein huntingtin.<sup>1</sup> The disease is characterized by motor impairment, cognitive deterioration, behavioral problems and progressive weight loss.<sup>1,2</sup> However, disturbed sleep is also a prominent feature of the disease, substantially impairing the quality of life of both patients and caregivers.<sup>3</sup> Polysomnographic and actigraphic findings in small groups of HD patients have documented an increased sleep onset latency, sleep fragmentation and frequent nocturnal awakenings, reduced sleep efficiency, delayed and shortened rapid eye movement (REM) sleep, increased periodic leg movements, as well as circadian rhythm disturbances.<sup>4-10</sup> However, the exact nature of sleep complaints as well as their association with other symptoms and signs of the disease have not yet been studied in large groups of HD patients or premanifest mutation carriers.

Recently, it was shown that the sleep-wake disturbances in HD patients are partly reproduced in the transgenic R6/2 mouse model of the disease.<sup>9</sup> Importantly, cognitive decline and decay of learning in R6/2 mice were alleviated by pharmacological imposition of sleep, suggesting that a similar strategy might be of benefit to HD patients.<sup>10,11</sup> Moreover, apart from affecting alertness, attention, memory and executive control, lack of sleep is also considered a risk factor for developing depression.<sup>12</sup> Conversely, recent clinical findings indicate that treatment of depressive symptoms may reduce sleep disruption in a number of neurodegenerative and extrapyramidal conditions, especially Alzheimer's and Parkinson's disease.<sup>13,14</sup> However, in order to provide rationale for the evaluation of comparable approaches in HD, it is first necessary to assess the association between sleep disruption and both cognitive impairment and depressive symptoms in HD patients.

Characterization of sleep problems in HD patients as well as the identification of contributing factors are of paramount importance for the design and implementation of novel therapeutic strategies. Therefore, the aims of the present study were to (1) delineate the nature of subjective sleep disturbances in HD patients and premanifest mutation carriers in comparison with non-mutation carrying control subjects, and (2) to assess the relation between sleep and various clinical characteristics, including cognitive impairment and depressive symptoms, in order to identify important predictors of sleep disturbances in HD patients. In addition, we evaluated the reliability and validity of the SCOPA-SLEEP questionnaire, a very short instrument that can assess both daytime sleepiness and night-time sleep,<sup>15</sup> in patients with HD.

## METHODS

### *Design and participants*

HD patients and premanifest mutation carriers were recruited from the outpatient clinic of the department of Neurology of the Leiden University Medical Center (LUMC) between June 2007 and December 2008. Control subjects were recruited over the same period and were randomly selected non-mutation carrying family members, partners or acquaintances of participating patients, employees at our department or their acquaintances. Exclusion criteria for both groups were the diagnosis of a preexistent primary sleep disorder or a disease of the central nervous system unrelated to HD. All patients and premanifest mutation carriers were

seen once or twice a year as part of their regular care. Three weeks before the scheduled yearly appointments two identical postal surveys containing standardized questionnaires were sent to these subjects. The forms in one of the surveys were completed by the patients or premanifest mutation carriers themselves (or by the primary caregivers on their behalf) while, whenever possible, the other survey had to be completed by a non-mutation carrying family member, the partner or an acquaintance. The patients and premanifest mutation carriers brought their survey to the scheduled appointment and simultaneously provided contact data for the control subject. When questionnaires were not fully completed or were not received within one month, subjects were reminded by phone. The study protocol was approved by the medical ethics committee of the LUMC and all participants provided written informed consent.

### *Measurement instruments*

The clinical condition of HD patients was assessed according to the motor, cognitive, behavioral and functional subscales of the Unified Huntington Disease Rating Scale (UHDRS).<sup>16</sup> Premanifest status was defined as a score of <5 on the UHDRS motor subscale. The following standardized instruments were used in all subjects to assess sleep disturbances and depressive symptoms: Epworth's Sleepiness Scale (ESS) [range (0-24)]<sup>17</sup>, the Pittsburgh Sleep Quality Index (PSQI) [range (0-21)]<sup>18</sup>, the SCOPA-SLEEP<sup>15</sup>, and the Beck Depression Inventory (BDI) [range (0-63)]. The ESS evaluates daytime sleepiness; subjects must rate the chance of dozing off under eight different situations.<sup>16</sup> The PSQI primarily evaluates night-time sleep, consisting of 19 self-rated questions and five questions rated by the bed partner or roommate. The latter five questions are used for clinical information only and are not tabulated in the scoring of the PSQI.<sup>18</sup> Scores are first grouped in seven domains which assess subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction.<sup>18</sup> The ESS and PSQI scales were included as they are frequently used and have previously been applied in small groups of HD patients.<sup>6</sup> The SCOPA-SLEEP questionnaire consists of two parts, one assessing daytime sleepiness (SCOPA-DS [range (0-18)]) and the other assessing night-time sleep (SCOPA-NS [range (0-15)]), and was originally developed for patients with Parkinson's disease.<sup>15</sup> However, since the items are not disease specific it can also be applied to other populations.<sup>15</sup> The SCOPA-SLEEP questionnaire was included as it is quick and easy to administer, and can assess sleep problems both during the day and night. In all questionnaires higher scores indicate more severe impairment.

### *Statistical Analysis*

Mean differences between HD patients, premanifest mutation carriers, partners and other controls were assessed using  $\chi^2$ -tests and one-way analysis of variance (ANOVA). Using multiple linear regression, group differences were also assessed after correction for differences in age, sex, body mass index (BMI), depressive symptoms and medication use (differentiating between sleep medications, antidepressants, neuroleptics and a rest group). In order to assess differences in usual bed and wake-up times (circular variables) between groups, we used pairwise Watson-Williams F-tests.<sup>19</sup> In HD patients, partial correlations, adjusting for age and sex, were used to assess the relation of each clinical variable — such as BMI, CAG repeat size and various UHDRS subscores — with sleep disturbances and timing. To identify which predictor variables were independently associated with sleep impairment in HD patients, we also used a stepwise regression procedure (an independent predictor



of sleep impairment is a variable that remains significantly associated with sleep impairment after adjustment for the effects of other significant predictor variables); this latter approach ensured minimization of type I error in identifying predictors of sleep impairment in HD. The reliability of all sleep scales in the patient group was assessed with Cronbach's  $\alpha$ . Using Pearson's correlation coefficients, the construct validity of the SCOPA-SLEEP in HD patients was assessed by comparing responses on its day and night subdomains with those on the ESS and PSQI scales, respectively. All data are presented as means  $\pm$  SD unless otherwise specified. All tests were two-tailed and values of  $p < 0.05$  were considered significant. Programming was performed in SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL) and Oriana version 2.02 for circular statistics (Kovach Computing Services, Wales, UK).

## RESULTS

### Participants

In total, 168 subjects participated in this study (Table 1). Of these 84 were mutation carriers (63 had manifest HD and 21 were premanifest), and 84 were non-mutation carrying subjects. As sleep problems of the mutation carriers may affect the sleep quality of their partners, non-mutation carriers were further divided into a partner group and a group consisting of other controls (Table 1); in the remainder of this paper we will refer to these two groups as partners and controls, respectively. The four groups did not significantly differ with respect to age, sex, and BMI (all  $p > 0.454$ ). Mutation carriers were significantly more depressed compared with non-carriers ( $p < 0.001$ ), however, no difference between manifest and premanifest HD subjects was found ( $p=0.106$ ). HD patients used significantly more antidepressants and neuroleptics compared to all other groups, while their use of sleep medication was significantly higher in comparison with controls only (Table 1).

**Table 1. Characteristics of the study participants**

	HD patients	Premanifest mutation carriers	Partners	Controls	<i>p</i> -value
<b>No. of participants</b>	63	21	21	63	-
<b>Sex (% men)</b>	46.0	42.9	47.6	41.3	0.972 <sup>†</sup>
<b>Age, mean (SD), yr</b>	48.5 (10.7)	44.4 (8.7)	48.3 (9.1)	47.4 (10.5)	0.454 <sup>‡</sup>
<b>BMI, mean (SD)</b>	25.3 (4.0)	25.5 (3.4)	25.5 (4.9)	25.3 (4.6)	0.991 <sup>‡</sup>
<b>BDI score, mean (SD)</b>	11.8 (9.4)	8.1 (8.4)	4.3 (5.7)	4.4 (4.6)	<0.001 <sup>‡</sup>
<b>Sleep medication, n (%)</b>	16 (25.4)	3 (14.3)	5 (23.8)	4 (6.3)	0.027 <sup>†</sup>
<b>Antidepressants, n (%)</b>	23 (36.5)	1 (4.8)	4 (19.0)	1 (1.6)	<0.001 <sup>†</sup>
<b>Neuroleptics, n (%)</b>	17 (27.0)	1 (4.8)	0	0	<0.001 <sup>†</sup>
<b>Other medication, n (%)</b>	29 (46.0)	9 (42.9)	11 (52.4)	16 (25.0)	0.105 <sup>†</sup>

<sup>†</sup>)  $\chi^2$ -tests.

<sup>‡</sup>) One-way ANOVAs.

**Legend:** BDI = Beck's Depression Inventory; BMI = Body Mass Index; HD = Huntington's disease

## Night-time sleep impairment

There were no significant differences in the global PSQI or SCOPA-NS scores between HD patients and premanifest subjects or between premanifest subjects and controls (Table 2). However, HD patients had significantly more night-time sleep impairment compared with controls as indicated by a higher percentage of patients having a PSQI score of 5 or higher (58.1% vs. 34.9%,  $p = 0.012$ ), as well as by higher mean scores on both the PSQI and SCOPA-NS scales (Table 2). The differences in mean PSQI and SCOPA-NS scores remained significant when corrected for age, sex and BMI ( $\beta = 1.369$ ,  $p = 0.037$  for PSQI, and  $\beta = 1.44$ ,  $p = 0.023$  for SCOPA-NS (unstandardized coefficients)). In order to assess the nature of differences in sleep disturbances between groups, we also compared scores on the seven subdomains of the PSQI questionnaire separately (Table 2). HD patients experienced significantly more daytime dysfunction compared with premanifest subjects ( $p = 0.009$ ). Moreover, compared with controls, HD patients had a significantly delayed sleep onset time, longer sleep duration, used more sleep medication and experienced more daytime dysfunction (Table 2). These findings remained significant when adjusted for age, sex and BMI (data not shown). Although there were no differences in usual bed time between groups, the usual wake-up time was significantly delayed in HD patients compared to both partners and controls (Table 2). There was also a trend towards a delayed wake-up time in premanifest subjects compared to controls ( $p=0.087$ ).

**Table 2. Sleep characteristics of the study population**

	HD patients <sup>†</sup>	Premanifest mutation carriers <sup>†</sup>	Partners <sup>†</sup>	Controls <sup>†</sup>
<b>PSQI global score</b>	6.0 (3.9) <sup>a</sup>	5.2 (3.7)	5.3 (4.4)	4.7 (3.0)
<b>Sleep quality</b>	0.9 (0.9)	0.9 (0.9)	0.9 (0.9)	0.8 (0.6)
<b>Sleep latency</b>	1.3 (1.1) <sup>a</sup>	0.9 (0.9 (1.0)	1.0 (1.1)	0.8 (0.9)
<b>Sleep duration</b>	0.5 (0.8) <sup>a, c</sup>	0.8 (0.9)	1.1 (0.8)	0.8 (0.8)
<b>Sleep efficiency</b>	0.8 (1.1)	0.8 (1.1)	0.6 (1.0)	0.6 (1.0)
<b>Sleep disturbances</b>	1.2 (0.6)	1.1 (0.5)	1.1 (0.4)	1.1 (0.4)
<b>Sleep medication</b>	0.5 (1.1) <sup>a</sup>	0.3 (0.6)	0.5 (1.1)	0.1 (0.5)
<b>Daytime dysfunction</b>	0.8 (0.8) <sup>a, b, c</sup>	0.4 (0.5)	0.3 (0.4)	0.5 (0.6)
<b>Usual bed time, hh:mm (circular SD)</b>	22:54 (01:53)	23:32 (01:58)	23:28 (00:41)	23:14 (01:51)
<b>Usual wake-up time, hh:mm (circular SE)</b>	08:11 (01:36) <sup>d, e</sup>	07:40 (01:17)	07:07 (00:51)	07:06 (01:07)
<b>SCOPA-NS</b>	4.5 (4.0) <sup>a</sup>	3.4 (3.4)	3.8 (4.2)	3.1 (2.6)
<b>ESS score</b>	4.5 (4.2)	4.5 (3.9)	5.1 (3.7)	4.8 (3.5)
<b>SCOPA-DS</b>	3.1 (3.6)	2.6 (3.5)	2.8 (3.2)	2.5 (2.6)

<sup>†</sup>) All data are indicated as mean total or subscores (SD)

<sup>a</sup>)  $p < 0.05$  with respect to controls (ANOVA)

<sup>b</sup>)  $p < 0.05$  with respect to premanifest mutation carriers (ANOVA)

<sup>c</sup>)  $p < 0.05$  with respect to partners (ANOVA)

<sup>d</sup>)  $p < 0.01$  with respect to controls (Watson-Williams F-test)

<sup>e</sup>)  $p < 0.01$  with respect to partners (Watson-Williams F-test)

**Legend:** ESS = Epworth's Sleepiness Scale; HD = Huntington's disease; PSQI = Pittsburgh Sleep Quality Index; SCOPA-DS = Scales for outcomes in Parkinson's disease-daytime sleep; SCOPA-NS = Scales for outcomes in Parkinson's disease-night-time sleep

As there were substantial differences in depressive symptoms between HD patients and controls, we repeated the analyses while correcting for the BDI score. After correction for the BDI score, differences in night-time sleep impairment between patients and controls became non-significant ( $p > 0.912$  for differences in both PSQI and SCOPA-NS scores). This relation remained similar when also corrected for use of sleep medication, antidepressants, neuroleptics, and other drugs.

### Daytime sleepiness

There were no significant differences in mean ESS or SCOPA-DS scores between groups (Table 2). Correction for age, sex, BMI, depression and medication use did not change the results. Only 12.7% of HD patients had a ESS score of 10 or higher as compared to 7.9% of controls ( $p = 0.560$ ).

### Reliability and validity of SCOPA-SLEEP in HD patients

The Cronbach's  $\alpha$  for SCOPA-NS was 0.89, which was higher than that for PSQI, with the corrected item-total correlations ranging from 0.44 to 0.88 (Table 3). Moreover, the scores on the SCOPA-NS and PSQI scales were highly correlated ( $r = +0.77$ ,  $p < 0.001$ ). Using the PSQI cutoff value of 4/5 to discriminate between good and bad sleepers as an external criterion for SCOPA-NS resulted in an area under the receiver operating characteristic curve of 0.85, with an optimal cutoff at 3/4, yielding a sensitivity of 0.81 and a specificity of 0.77. The Cronbach's  $\alpha$  for SCOPA-DS was 0.85, which was also higher than that of the comparable instrument ESS (Table 3). However, item 4 on the SCOPA-DS questionnaire had a corrected item-total correlation of zero as there were no HD patients who reported falling asleep while talking (Table 3), indicating that this question is not suitable for an HD population. The other corrected item-total correlations of SCOPA-DS were relatively high and ranged from 0.61 to 0.86 (Table 3). There was also a strong correlation between scores on the SCOPA-DS and ESS questionnaires ( $r = +0.75$ ,  $p < 0.001$ ). Applying the ESS cutoff value of 9/10 to detect excessive daytime sleepiness as an external criterion for SCOPA-DS led

**Table 3. Reliability of sleep scales in HD patients**

	Cronbach's $\alpha$	Corrected item-total correlations
<b>SCOPA-NS</b>	0.89	0.44 – 0.88
1: Difficulty falling asleep		0.79
2: Been awake too often		0.81
3: Lying awake too long		0.88
4: Waking too early		0.44
5: Had too little sleep		0.76
<b>PSQI</b>	0.72	-0.01 – 0.74
<b>SCOPA-DS</b>	0.85	0.00 – 0.86
1: Falling asleep unexpectedly		0.76
2: Falling asleep while sitting		0.86
3: Falling asleep while watching TV		0.61
4: Falling asleep while talking		0.00
5: Difficulty staying awake		0.74
6: Falling asleep considered a problem		0.66
<b>ESS</b>	0.77	0.69 – 0.77

**Legend:** ESS = Epworth's Sleepiness Scale; HD = Huntington's disease; PSQI = Pittsburgh Sleep Quality Index; SCOPA-DS = Scales for outcomes in Parkinson's disease-daytime sleep; SCOPA-NS = Scales for outcomes in Parkinson's disease-night-time sleep

to an area under the receiver operating characteristic curve of 0.92, with an optimal cutoff at 3/4, yielding a sensitivity of 1.0 and a specificity of 0.78.

### *Predictors of night-time sleep and daytime sleepiness in HD patients*

As SCOPA-SLEEP appeared more reliable than PSQI and ESS for application in HD patients, we used scores on SCOPA-NS and SCOPA-DS to assess the relation between various clinical measures and sleep impairment in HD patients. Of all clinical measures tested, only the BDI score significantly correlated with both night-time sleep impairment and daytime sleepiness (Table 4). Moreover, stepwise regression also identified BDI score as the only independent predictor of night-time sleep impairment, accounting for 19% of the variation in sleep disturbances. However, BDI score was not independently associated with daytime sleepiness in HD patients (Table 4).

**Table 4. Correlates of night-time sleep impairment and daytime sleepiness in HD patients**

	SCOPA-NS score		SCOPA-DS score	
	Correlation <sup>†</sup> (r ; p-value)	Stepwise regression <sup>†</sup> (effect; SE)	Correlation <sup>†</sup> (r ; p-value)	Stepwise regression <sup>†</sup> (effect (SE))
<b>BMI, kg/m<sup>2</sup></b>	-0.11 (0.404)	-	-0.09 (0.494)	-
<b>CAG repeat no.</b>	0.23 (0.094)	-	0.125 (0.363)	-
<b>TFC score</b>	0.01 (0.956)	-	0.05 (0.691)	-
<b>Motor score</b>	0.02 (0.876)	-	-0.25 (0.058)	-
<b>Behavioral score</b>	-0.01 (0.943)	-	0.01 (0.936)	-
<b>Cognitive score</b>	-0.07 (0.685)	-	0.187 (0.282)	-
<b>BDI score</b>	0.38 (0.002)**	0.16 (0.071)*	0.27 (0.033)*	-

<sup>†</sup>) All results are corrected for age and sex.

\*) p < 0.05; \*\*) p < 0.01.

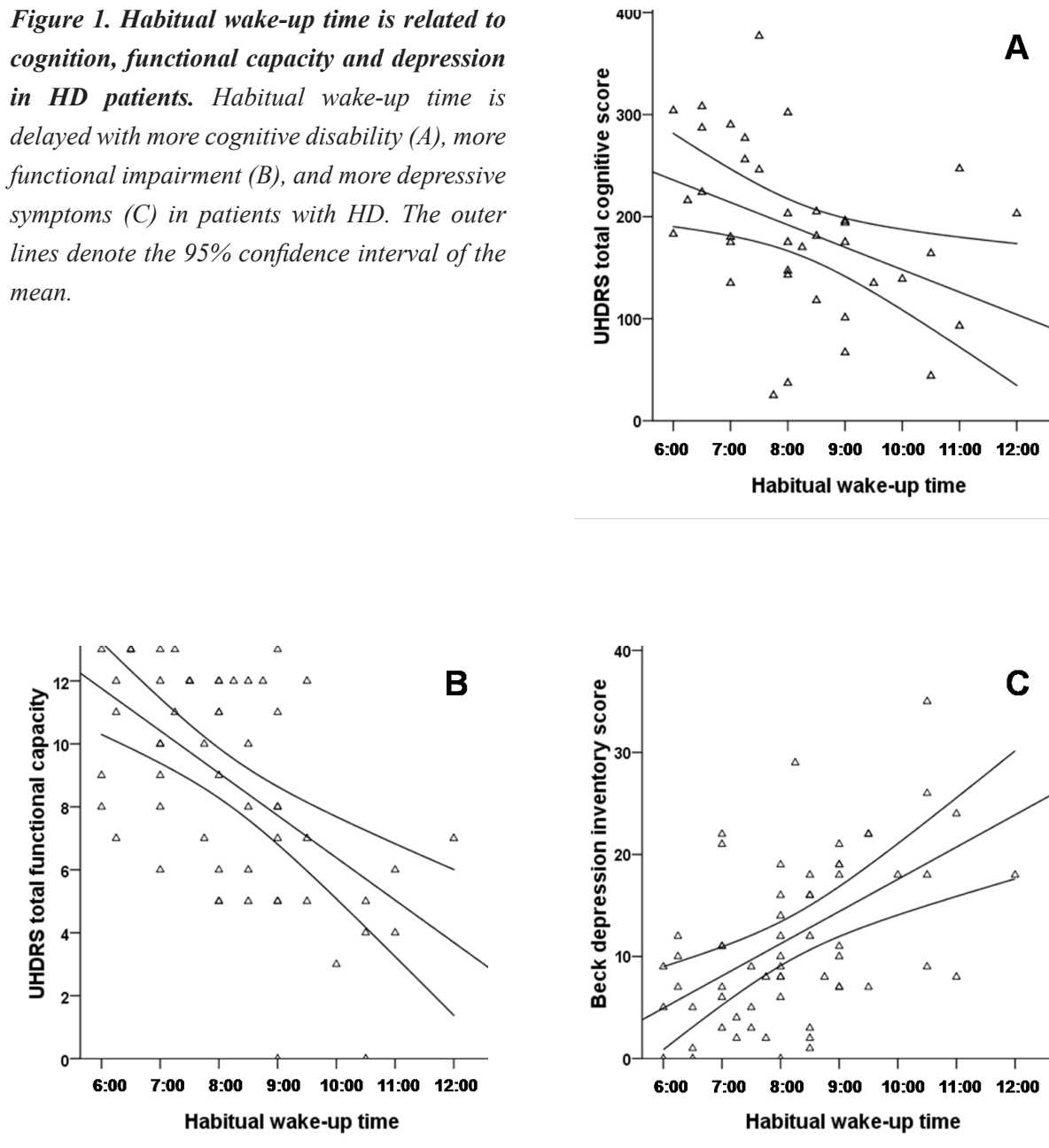
**Legend:** BDI = Beck's Depression Inventory; BMI = Body Mass Index; HD = Huntington's disease; ESS = Epworth's Sleepiness Scale; HD = Huntington's disease; PSQI = Pittsburgh Sleep Quality Index; SCOPA-DS = Scales for outcomes in Parkinson's disease-daytime sleep; SCOPA-NS = Scales for outcomes in Parkinson's disease-night-time sleep; TFC = Total Functional Capacity

### *Circadian timing of sleep and cognition in HD patients*

In order to assess whether circadian timing of sleep could affect cognitive functioning in HD patients, we examined the associations between usual bed and wake-up times and indices of cognitive performance in these subjects. UHDRS cognitive scores were available in 37 HD patients. Whereas the usual bed time was not significantly associated with the UHDRS cognitive scores, there were strong inverse associations between the usual wake-up time and the total cognitive score ( $r=-0.43$ ,  $p=0.011$ ; Figure 1A), and scores on the symbol digit modalities test ( $r=-0.45$ ,  $p=0.003$ ), and Stroop's colour naming ( $r=-0.47$ ,  $p=0.005$ ), word reading ( $r=-0.40$ ,  $p=0.010$ ), and interference ( $r=-0.56$ ,  $p=0.001$ ) tests. There was also a trend for the association between wake-up time and the verbal fluency test score ( $r=-0.30$ ,  $p=0.076$ ). In addition, the wake-up time was progressively

delayed with more severe total functional impairment ( $r=-0.54$ ,  $p<0.001$ ; Figure 1B) and more depressive symptoms ( $r=0.47$ ,  $p<0.001$ ; Figure 1C), but not with motor score, mutant CAG repeat or BMI (all  $p \geq 0.083$ ). As cognitive impairment and total functional capacity were highly correlated ( $r=+0.71$ ,  $p<0.001$ ), it was not possible to assess the association between wake-up time and cognitive score independent of functional impairment. Importantly, however, the association between wake-up time and total cognitive score remained significant even after correction for the BDI score ( $r=-0.34$ ,  $p=0.047$ ).

**Figure 1. Habitual wake-up time is related to cognition, functional capacity and depression in HD patients.** Habitual wake-up time is delayed with more cognitive disability (A), more functional impairment (B), and more depressive symptoms (C) in patients with HD. The outer lines denote the 95% confidence interval of the mean.



## DISCUSSION

To our knowledge, this is largest cohort of HD patients and premanifest mutation carriers whose subjective sleep quality and daytime somnolence have been systematically assessed in relation to clinical symptoms and signs. Our findings indicate that while night-time sleep impairment is indeed more prevalent in HD patients, daytime sleepiness appears unlikely to be a major issue in HD. We also show that depression is the most important clinical predictor of sleep impairment in HD patients. Moreover, our findings suggest a delayed sleep phase syndrome (DSPS)-like circadian rhythm disorder in HD patients which appears to be associated with lower cognitive performance. These findings indicate that treatment of depressive symptoms and re-entrainment of the circadian rhythms could be evaluated as measures to enhance both sleep and cognitive functioning in HD patients.

In line with previous findings from a number of smaller-scale studies,<sup>4-10</sup> we found that night-time sleep disturbances are almost twice as common in HD patients compared to controls. Importantly, while the usual bed time did not differ between the groups, sleep onset latency as well as the usual wake-up time were both significantly delayed in HD patients, suggesting a phase-shift in the circadian sleep/wake cycle towards later hours. This finding is particularly interesting as recently we also found a delayed onset of the diurnal melatonin rise in a group of early-stage HD patients,<sup>20</sup> and is likewise suggestive of a DSPS-like circadian rhythm disorder. The pathophysiological basis of DSPS is presumed to lie within a slower endogenous clock with an abnormally long intrinsic circadian periodicity, resulting in a delayed phase position of the overt circadian rhythms, including those of melatonin and cortisol.<sup>21</sup> Interestingly, recently we reported an increased rate of early day cortisol production in HD patients as well, which may also be a manifestation of delayed circadian rhythms in HD.<sup>22</sup> Circadian rhythm disturbances in HD are likely to stem directly from pathology within the suprachiasmatic nucleus molecular oscillation, resulting from either the toxic effects of mutant huntingtin locally and/or arising from dysfunction of brain circuitry afferent to the suprachiasmatic nucleus,<sup>9,10</sup> but additional studies are needed to pinpoint the exact underlying cause. Although a delayed sleep onset has been described previously in smaller groups of HD patients<sup>7,23,24</sup>, others have reported an earlier sleep onset and an advanced sleep phase.<sup>4</sup> However, the latter study included only 25 HD subjects (including 2 premanifest mutation carriers) and assessed bed and wake-up times during a single occasion in a laboratory setting, whereas we inquired about the usual bed and wake-up times in the previous month which are likely to be more representative of the habitual sleeping times.

Restoration of circadian rhythms by pharmacological imposition of sleep has been shown to improve cognition in R6/2 mice, suggesting that a similar strategy may be beneficial to HD patients.<sup>10,11</sup> As our findings are reminiscent of a DSPS-like phenotype in HD patients that is associated with cognitive impairment, another approach that may be evaluated in these patients is melatonin and/or bright light treatment at the appropriate times so as to phase advance the clock.<sup>21,25,26</sup> The administration of melatonin at the subjective dusk, and the use of bright light at the subjective dawn and avoidance of light in the subjective evening, could be used to phase advance the clock.<sup>21,25</sup> However, we could not detect a significant association between cognitive performance and night-time sleep disturbances in HD patients, suggesting that circadian rhythm alterations rather than sleep impairment *per se* are related to cognitive disability in these subjects. Nevertheless, the lack of an association



between night-time sleep and cognitive scores could also be due to the use of subjective measures of sleep quality in this study. As particularly REM sleep and deep sleep are thought to be implicated in neurobehavioral and cognitive performance,<sup>27,28</sup> and abnormalities have been reported in both of these sleep stages in HD,<sup>4,7,29</sup> future studies should focus on the exploration of the association between polysomnographic sleep measures and cognitive functioning in HD patients.

Depressed mood is highly prevalent in HD patients, with estimates ranging from 33 to 69%, and is an important determinant of their quality of life.<sup>30,31</sup> Here we demonstrate that depressed mood is also the most important clinical predictor of sleep disturbances in HD patients. This finding extends upon a previous report<sup>6</sup> by showing that the association between depressive symptoms and sleep impairment is independent from other symptoms and signs of the disease including motor, cognitive and functional impairment. However, due to the bidirectional association between depression and sleep disturbances, it is difficult to differentiate between cause and effect.<sup>32</sup> Depression is identified as the most frequent cause of chronic insomnia in both clinical and epidemiological studies,<sup>33,34</sup> and as many as three quarters of individuals with DSPS have a past or current history of depression.<sup>35</sup> Conversely, a number of longitudinal studies indicate that insomnia is a risk factor for developing both first-onset and recurrent depression.<sup>32,36,37</sup> The co-morbidity of depression and insomnia may be explained by changes in systems that are involved in both mood and sleep regulation, especially the serotonergic and noradrenergic systems.<sup>38</sup> Therefore, comprehensive treatment of depressive symptoms could help to resolve sleep complaints, whereas treatment of sleep problems and circadian rhythm alterations could help to bring about remission of depressive symptoms.<sup>39</sup> Thus, simultaneous treatment of depressive symptoms, sleep problems and circadian rhythm changes in HD patients should be evaluated in future studies as the effects may be synergistic with regard to both mood and sleep improvement.

In this study we also assessed the performance of the SCOPA-SLEEP questionnaire in HD patients. Both its night and day subdomains revealed a high degree of internal consistency in this population. Moreover, the correlation with other scales that assess similar constructs was strong, indicating good construct validity. As our findings primarily indicate night-time sleep problems in HD patients, particularly SCOPA-NS may prove to be valuable for application in these patients as it takes very little time to complete while its reliability and consistency in this population are actually higher than those of more extensive and time-consuming scales such as the PSQI.

In conclusion, our findings indicate mainly night-time sleep disturbances in HD patients and are suggestive of a DSPS-like phenotype associated with lower cognitive performance. Moreover, we identified depression as the principal predictor of sleep impairment in HD patients. As the mechanisms regulating sleep, mood and circadian rhythms show considerable overlap, comprehensive treatments aimed at alleviation of depressive symptoms and sleep impairment, as well as re-entrainment of circadian rhythms in HD patients may have synergistic effects and should be evaluated in future studies.



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### **Autonomic symptoms in patients and premanifest mutation carriers of Huntington's disease**

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## ABSTRACT

*Objective:* Although autonomic function tests have revealed abnormalities of the autonomic nervous system in Huntington's disease (HD), autonomic symptoms and their association with other symptoms and signs of HD have not yet been assessed in large groups of patients or premanifest mutation carriers. We therefore aimed to delineate the characteristics and correlates of autonomic symptoms in HD. *Subjects & methods:* Using the SCOPA-AUT and Beck Depression Inventory questionnaires, autonomic symptoms and depressed mood were assessed in 63 HD patients, 21 premanifest mutation carriers and 85 controls. The Unified Huntington's Disease Rating Scale was used to assess other HD symptoms and signs. *Results:* Relative to controls, HD patients experienced significantly more gastrointestinal, urinary, cardiovascular and, in men, sexual problems. The most prevalent symptoms were swallowing difficulties, erection and ejaculation problems, dysphagia, sialorrhea, early abdominal fullness, straining for defecation, fecal and urinary incontinence, urgency, incomplete bladder emptying, and light-headedness while standing. Premanifest mutation carriers experienced significantly more swallowing difficulties and light-headedness on standing up compared with controls. In HD patients, autonomic symptoms were associated with a greater degree of functional disability, more severe depression and antidepressant drugs use; however, depression was the only independent predictor of autonomic dysfunction. *Conclusions:* Autonomic symptoms are highly prevalent in HD patients and may even precede the onset of motor signs. Moreover, autonomic dysfunction is related to functional disability and depression in HD.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat sequence in the gene encoding the protein huntingtin.<sup>1</sup> The disease is characterized by motor impairment, cognitive deterioration, behavioral problems and progressive weight loss.<sup>1,2</sup> Although less well-known, autonomic nervous system (ANS) dysfunction can also accompany HD.<sup>3</sup> Indeed, standardized ANS tests, including the blood pressure response to sustained handgrip,<sup>4,5</sup> orthostatic blood pressure test,<sup>4,7</sup> sympathetic skin response,<sup>4,6,7</sup> the pupillary light reflex latency,<sup>4</sup> and heart rate variability assessment at rest and during various maneuvers,<sup>4,8</sup> have revealed abnormalities in both the sympathetic and parasympathetic branches of the ANS in HD.

Autonomic symptoms such as hyperhidrosis, micturition and swallowing difficulties,<sup>9-11</sup> sexual dysfunction,<sup>12</sup> as well as complaints suggestive of orthostatic intolerance<sup>13,14</sup> have been reported in small groups of HD patients. Moreover, although autonomic symptoms are thought to be most prominent in the advanced stages of the disease,<sup>15,16</sup> complaints of possible autonomic origin such as dizziness after standing up, excessive perspiration and tachycardia have also been described in mildly disabled HD patients and even in otherwise asymptomatic mutation carriers.<sup>5</sup> However, the extent of autonomic complaints as well as their association with other symptoms and signs of the disease have not yet been studied in large groups of HD patients or premanifest mutation carriers.

Therefore, the objectives of the present study were to (1) delineate the characteristics and frequency of autonomic symptoms in HD patients and premanifest mutation carriers in comparison with non-mutation carrying control subjects, and (2) to assess the relation between autonomic symptoms and various clinical characteristics in order to identify important predictors of autonomic problems in HD patients.

## METHODS

### *Design and participants*

HD patients and premanifest mutation carriers were successively recruited from the outpatient clinic of the department of Neurology of the Leiden University Medical Center (LUMC) between June 2007 and December 2008. Control subjects were recruited over the same period and were randomly selected non-mutation carrying family members, partners or acquaintances of participating patients, employees at our department or their acquaintances. Exclusion criteria for both groups were the diagnosis of a primary ANS disorder or a disease of the central nervous system unrelated to HD. All patients and premanifest mutation carriers were seen once or twice a year as part of their regular care. Three weeks before the scheduled annual appointments two identical postal surveys containing standardized questionnaires were sent to these subjects. The forms in one of the surveys were completed by the patients or premanifest mutation carriers themselves (or by the primary caregivers on their behalf) while, whenever possible, the other survey had to be completed by a non-mutation carrying family member, the partner or an acquaintance. The patients and premanifest mutation carriers brought their survey to the scheduled appointment and simultaneously provided contact data for the control subject. When questionnaires were not fully completed or were not received within one month, subjects were reminded

by phone. The study protocol was approved by the medical ethics committee of the LUMC and all participants gave written informed consent.

### *Measurement Instruments*

The clinical diagnosis of HD was made by a neurologist specialized in movement disorders (R.A.C.R.). The clinical condition of HD patients was assessed according to the motor, cognitive, behavioral and functional subscales of the Unified Huntington Disease Rating Scale (UHDRS).<sup>17</sup> Premanifest status was defined as a score of  $<5$  on the UHDRS motor scale. Symptoms of depression were evaluated by the Beck Depression Inventory (BDI).<sup>18</sup> Autonomic symptoms were assessed using the SCOPA-AUT questionnaire.<sup>19</sup> Although the SCOPA-AUT questionnaire was originally developed for patients with Parkinson's disease, the items cover a broad area of autonomic domains that may also be affected in HD.<sup>3</sup> The SCOPA-AUT questionnaire was selected as it is quick and easy to administer and has already been demonstrated to have a good test-retest reliability in a Dutch population (both Parkinson's disease patients and controls).<sup>19,20</sup> The SCOPA-AUT consists of 25 items assessing the following domains: gastrointestinal (7), urinary (6), cardiovascular (3), thermoregulatory (4), pupillomotor (1), and sexual (2 items for men and 2 items for women) dysfunction. The frequency of the problem was evaluated with four response options ranging from 0 ("never") to 3 ("often"). The urinary and sexual regions have an additional response option, to indicate whether a subject used a catheter or had not been sexually active.<sup>19</sup> In order to simplify comparison, the total and domain scores were all converted into relative scores with a range of 0 to 100, with higher scores indicating more severe impairment. In addition, to assess the relative frequency of each specific autonomic symptom in HD, the percentages within each participating group with item scores of  $\geq 1$  were compared.

### *Statistical Analysis*

Mean differences between HD patients, premanifest mutation carriers, partners and other controls were assessed using  $\chi^2$ -tests and one-way analysis of variance (ANOVA) after a square-root transformation in case of non-normal distribution. Tukey's test (in case of equal variances) or Dunnett's T3 test (in case of unequal variances) was used for post-hoc analysis. In HD patients, partial correlations were used to assess the relation of each clinical variable — such as various UHDRS subscores and medication use (differentiating between sleep medications, antidepressants, neuroleptics and a rest group) — with the total autonomic symptom score, while correcting for the effects of age and sex. To identify which predictor variables were independently associated with the total autonomic score in HD patients, we also used a forward stepwise regression procedure with entry and removal probabilities for F of 0.05 and 0.10, respectively. An independent predictor of autonomic dysfunction is a variable that remains significantly associated with autonomic symptom severity after adjustment for the effects of other significant predictor variables. Additionally, in HD patients, Spearman's correlation coefficient was used to assess the association between motor impairment and each domain score separately, as well as to assess the correlates of sexual dysfunction. All tests were two-tailed and values of  $p < 0.05$  were considered significant. The analyses were performed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL).



## RESULTS

### Participants

In total, 169 subjects participated in this study (Table 1). Of these 84 were mutation carriers (63 had manifest HD and 21 were premanifest), and 85 were non-mutation carrying subjects. As autonomic problems, such as sexual dysfunction, of the mutation carriers may affect their partners, non-mutation carriers were further divided into a partner group and a group consisting of other controls (Table 1). The four groups did not differ with respect to age, sex, and BMI (all  $p > 0.453$ ). Mutation carriers were significantly more depressed compared with non-carriers ( $p < 0.001$ ), but the degree of depression did not differ between manifest and premanifest HD subjects ( $p=0.10$ ). HD patients used significantly more antidepressant and neuroleptic drugs than the other groups (Table 1).

**Table 1. Characteristics of the study participants**

	HD patients	Premanifest mutation carriers	Partners	Controls	<i>p</i> -value
<b>No. of participants</b>	63	21	21	64	-
<b>Sex (% men)</b>	29/34 (46.0)	9/12 (42.9)	10/11 (47.6)	27/37 (42.2)	0.959†
<b>Age, mean (SD), yr</b>	48.5 (10.7)	44.4 (8.7)	48.3 (9.1)	47.4 (10.4)	0.453‡
<b>BMI, mean (SD)</b>	25.3 (4.0)	25.5 (3.4)	25.5 (4.9)	25.3 (4.6)	0.997‡
<b>BDI score, mean (SD)</b>	11.8 (9.4)	8.1 (8.4)	4.3 (5.7)	4.6 (4.8)	<0.001‡
<b>Sleep medication, n (%)</b>	16 (25.4)	3 (14.3)	5 (23.8)	5 (7.8)	0.052†
<b>Antidepressants, n (%)</b>	23 (36.5)	1 (4.8)	4 (19.0)	1 (1.6)	<0.001†
<b>Neuroleptics, n (%)</b>	17 (27.0)	1 (4.8)	0	0	<0.001†
<b>Other medication, n (%)</b>	29 (46.0)	9 (42.9)	11 (52.4)	16 (25.0)	0.138†

†)  $\chi^2$ -tests.

‡) One-way ANOVAs.

Legend: BDI = Beck's Depression Inventory; BMI = Body Mass Index; HD = Huntington's disease

### Autonomic symptoms: domains

The SCOPA-AUT items regarding sexual function were answered with 'not applicable' by a substantial minority in all groups, therefore, these items were not included for the calculation of the total score. Overall, patients with HD reported significantly more autonomic symptoms compared with both partners and controls as indicated by a higher total SCOPA-AUT score (Table 2). However, the total SCOPA-AUT score did not differ between HD patients and premanifest mutation carriers ( $p=0.33$ ). Compared with partners and controls, HD patients experienced significantly more gastrointestinal, cardiovascular and, in men, sexual problems. HD patients also reported significantly more gastrointestinal and male sexual problems than premanifest mutation carriers (Table 2). In general, the autonomic domain scores of premanifest mutation carriers were in between those of HD patients and non-mutation carriers.

**Table 2. Autonomic symptoms severity (range 0 to 100) and frequency (% with an item score  $\geq 1$ ) in the study population.**

	<b>HD patients</b>	<b>Premanifest</b>	<b>Partners</b>	<b>Controls</b>	<b>p-value<sup>1</sup></b>
<b>Total score (median, IQR)<sup>2</sup></b>	<b>16 (10-24)<sup>a,c</sup></b>	<b>14 (7-18)</b>	<b>7 (4-12)</b>	<b>10 (6-14)</b>	<b>&lt;0.001<sup>**</sup></b>
<b>Gastrointestinal domain (median, IQR)<sup>2</sup></b>	<b>14 (5-19)<sup>a,b,c</sup></b>	<b>5 (2-10)<sup>c</sup></b>	<b>0 (0-5)</b>	<b>5 (0-10)</b>	<b>&lt;0.001<sup>**</sup></b>
Swallowing/choking (%)	71 <sup>a,b,c</sup>	48 <sup>d,e</sup>	5 <sup>f</sup>	16	<0.001 <sup>**</sup>
Sialorrhea (%)	32 <sup>a,b,c</sup>	0	0	11	<0.001 <sup>**</sup>
Dysphagia (%)	35 <sup>a,c</sup>	14	5	8	<0.001 <sup>**</sup>
Early abdominal fullness (%)	32 <sup>a</sup>	24	25	16	0.206
Constipation (%)	11	10	5	9	0.882
Straining for defecation (%)	37 <sup>c</sup>	33	10	27	0.134
Fecal incontinence (%)	16 <sup>a</sup>	5	0	3	0.021 <sup>*</sup>
<b>Urinary domain (median, IQR)<sup>2</sup></b>	<b>22 (11-39)</b>	<b>17 (11-22)</b>	<b>11 (7-17)</b>	<b>17 (11-26)</b>	<b>0.066<sup>*</sup></b>
Urgency (%)	44 <sup>a,b,c</sup>	19	5	27	0.003 <sup>**</sup>
Urinary incontinence (%)	32 <sup>a</sup>	14	10	14	0.038 <sup>*</sup>
Incomplete emptying (%)	27 <sup>c</sup>	14	5	20	0.164
Weak stream of urine (%)	29	14	10	20	0.264
Frequency (%)	68	81	70	78	0.507
Nocturia (%)	78	81	70 <sup>f</sup>	89	0.189
<b>Cardiovascular domain (median, IQR)<sup>2</sup></b>	<b>0 (0-11)<sup>a</sup></b>	<b>0 (0-11)</b>	<b>0 (0-8)</b>	<b>0 (0-0)</b>	<b>0.047<sup>*</sup></b>
Light-headed when standing up (%)	33 <sup>a</sup>	38 <sup>d</sup>	25	17	0.123
Light-headed when standing for some time (%)	16 <sup>a</sup>	5	0	3	0.021 <sup>*</sup>
Syncope in the past 6 months (%)	5	0	0	0	0.165
<b>Thermoregulatory domain (median, IQR)<sup>2</sup></b>	<b>17 (0-25)</b>	<b>17 (0-29)</b>	<b>8 (0-25)</b>	<b>17 (0-25)</b>	<b>0.751</b>
Hyperhidrosis during the day (%)	37	43	30	42	0.742
Hyperhidrosis during the night (%)	41	52	45	48	0.784
Cold intolerance (%)	38	33	25	27	0.491
Heat intolerance (%)	44	38	35	33	0.590
<b>Pupillomotor domain (median, IQR)<sup>2</sup></b>	<b>0 (0-0)</b>	<b>0 (0-33)</b>	<b>0 (0-33)</b>	<b>0 (0-33)</b>	<b>0.855</b>
Over-sensitive for bright light (%)	24	33	35	30	0.711
<b>Sexual domain: men (median, IQR)<sup>2</sup></b>	<b>33 (0-58)<sup>a,b,c</sup></b>	<b>0 (0-8)</b>	<b>0 (0-0)</b>	<b>0 (0-0)</b>	<b>0.004<sup>**</sup></b>
Erection problem (%)	67 <sup>a,b,c</sup>	22	11	22	<0.001 <sup>**</sup>
Ejaculation problem (%)	59 <sup>a,b,c</sup>	0	11	19	<0.001 <sup>**</sup>
'Not applicable' (%)	21	0	10	7	0.275
<b>Sexual domain: women (median, IQR)<sup>2</sup></b>	<b>0 (0-17)</b>	<b>8 (0-42)</b>	<b>0 (0-17)</b>	<b>8 (0-29)</b>	<b>0.562</b>
Women: Vaginal lubrication (%)	53	42	55	49	0.904
Women: Problem with orgasm (%)	53	67	46	51	0.755
'Not applicable'	38	33	36	24	0.633

<sup>1</sup>) Differences in means were assessed by one-way analysis of variance (after a square-root transformation in case of non-normal distribution) with Tukey's test (in case of equal variances) or Dunnett's T3 test (in case of unequal variances) for post-hoc analysis. Differences in proportions were assessed by the  $\chi^2$ -test. \* p<0.05; \*\* p<0.01

<sup>2</sup>) To simplify comparison, the total and domain scores were all converted into relative scores with a range of 0 to 100. IQR = interquartile range

<sup>a</sup>) Patients vs. controls (p<0.05)

<sup>b</sup>) Patients vs. premanifest mutation carriers (p<0.05)

<sup>c</sup>) Patients vs. partners (p<0.05)

<sup>d</sup>) Premanifest mutation carriers vs. control (p<0.05)

<sup>e</sup>) Premanifest mutation carriers vs. partners (p<0.05)

<sup>f</sup>) Partners vs. controls (p<0.05)

### *Autonomic symptoms: frequency*

The largest differences between HD patients relative to controls existed for the frequency of swallowing/choking (71 vs. 16%), erection problems (67 vs. 22%), ejaculation problems (59 vs. 19%), dysphagia (35 vs. 8%), and sialorrhea (32 vs. 11%) (Table 2). Other relatively prevalent autonomic symptoms in HD patients were early abdominal fullness, straining for defecation, fecal and urinary incontinence, urgency, incomplete bladder emptying, and light-headedness while standing (up). Of these symptoms, only swallowing difficulties, sialorrhea, urgency, and erection and ejaculation problems were also more frequent in HD patients compared to premanifest mutation carriers. Premanifest mutation carriers experienced significantly more swallowing difficulties and light-headedness on standing up compared with controls (Table 2).

### *Predictors of autonomic dysfunction in HD patients*

Only UHDRS functional indices, i.e. total functional capacity, functional assessment and independence score, were significantly associated with the total autonomic dysfunction score in HD patients (Table 3). The total score also positively correlated with depressive symptoms as well as use of antidepressant drugs (both  $p < 0.045$ ). Moreover, stepwise regression identified depressive symptoms as the only independent predictor of autonomic dysfunction, accounting for 32% of the variation (Table 3). Motor impairment was not significantly associated with any domain score in HD patients (all  $p \geq 0.171$ ).

**Table 3. Correlates of autonomic dysfunction in HD patients**

	<b>SCOPA-AUT total score</b>	
	<b>Correlation<sup>†</sup> (r ; p-value)</b>	<b>Stepwise regression<sup>†</sup> (effect; SE)</b>
<b>Motor score</b>	-0.00 (0.975)	-
<b>TFC</b>	-0.37 (0.006) **	-
<b>Functional assessment</b>	-0.35 (0.008) **	-
<b>Independence score</b>	-0.28 (0.038) *	-
<b>Cognitive score</b>	-0.10 (0.574)	-
<b>Behavior score</b>	0.04 (0.796)	-
<b>BDI</b>	0.57 (<0.001) **	0.46 (0.18) **
<b>BMI</b>	0.22 (0.086)	-
<b>Normal CAG repeat size</b>	0.01 (0.923)	-
<b>Mutant CAG repeat size</b>	0.03 (0.833)	-
<b>Sleeping medication</b>	0.23 (0.081)	-
<b>Antidepressants</b>	0.26 (0.045) *	-
<b>Neuroleptics</b>	0.13 (0.303)	-
<b>Rest</b>	-0.04 (0.735)	-

<sup>†</sup>) All results are corrected for age and sex.

\*)  $p < 0.05$ ; \*\*  $p < 0.01$ .

**Legend:** BDI = Beck's Depression Inventory; BMI = Body Mass Index; HD = Huntington's disease; HD = Huntington's disease; SCOPA-AUT = Scales for outcomes in Parkinson's disease-autonomic symptoms; TFC = Total Functional Capacity

As the items regarding sexual function were gender specific and not included in the total SCOPA-AUT score, we assessed this domain separately. In male HD patients, sexual dysfunction was significantly associated with all UHDRS functional indices ( $r$  from -0.49 to -0.61,  $p \leq 0.028$ ), BMI ( $r = +0.54$ ,  $p = 0.012$ ), depressive symptoms ( $r = +0.52$ ,  $p = 0.017$ ), and use of antidepressants ( $r = +0.54$ ,  $p = 0.013$ ). In female HD patients, however, sexual dysfunction was only significantly related to the behavioural score ( $r = -0.48$ ,  $p = 0.045$ ).

## DISCUSSION

To our knowledge, this is the largest cohort of HD patients and premanifest mutation carriers whose autonomic symptoms have been systematically assessed in relation to other clinical symptoms and signs. We found a range of autonomic symptoms in HD patients, particularly in the gastrointestinal, urinary, cardiovascular and male sexual domains, some of which were also present in premanifest mutation carriers. These findings indicate that, autonomic symptoms are highly prevalent in HD patients and may even precede the onset of motor signs. In addition, we found that a greater degree of autonomic dysfunction in HD patients was associated with more functional disability as well as more depressive symptoms.

The most prominent complaints in HD patients concerned the gastrointestinal tract and included swallowing difficulties, dysphagia and sialorrhea. These symptoms may partly be accounted for by skeletal muscle incoordination due to striatal pathology.<sup>10</sup> However, the absence of an association between gastrointestinal symptoms and UHDRS motor score, as well as the relatively high prevalence of swallowing difficulties even in premanifest mutation carriers suggest that ANS dysfunction is also likely to contribute to gastrointestinal problems in HD. This notion is further supported by the comparatively high prevalence of other symptoms in HD patients, including early abdominal fullness, straining for defecation, urgency, incomplete bladder emptying, and postural dizziness, which cannot be ascribed to motor impairment alone. Nevertheless, although our findings suggest that ANS dysfunction is likely to contribute to the various symptoms enlisted in Table 2, we cannot exclude other potential contributing factors such as endocrine and peripheral abnormalities.<sup>3,21</sup> Therefore, further studies simultaneously applying both subjective and objective measures, such as the composite autonomic scoring scale,<sup>22</sup> should be undertaken to more accurately delineate the role of ANS dysfunction in the pathogenesis of the aforementioned symptoms in HD.

Interestingly, while erection and ejaculation problems were very prominent in male HD patients, sexual dysfunction was not obvious in female patients. However, relatively more HD patients than controls, and relatively more females than males, answered ‘not applicable’ to one or both of the sexual items in the questionnaire, which could indicate sexual hypofunction.<sup>12</sup> Our results may therefore represent underestimates of the actual prevalence of sexual dysfunction in, particularly female, HD patients.

Autonomic dysfunction was associated with both functional disability and depression in HD patients. Due to

the cross-sectional nature of our study, it is impossible to differentiate between cause and effect. However, autonomic symptoms are unlikely to be secondary to functional impairment, as by definition, autonomic functions are not under voluntary control. It is rather more likely that symptoms such as dysphagia, sialorrhea, and fecal and urinary incontinence could lead to social embarrassment, isolation and a negative impact on daily functioning. On the other hand, the relation between autonomic dysfunction and depression, which is also found in other patient populations,<sup>20</sup> is harder to explain since depression itself can be accompanied by several vegetative symptoms such as loss of libido.<sup>23</sup> In fact, depression was the only independent predictor of autonomic dysfunction in HD patients, indicating that its effect on autonomic symptoms is independent of, e.g., functional disability. Although antidepressant use was correlated with the extent of autonomic complaints in HD patients, adjustment for the severity of depressive symptoms rendered this relation insignificant, suggesting that the association between depression and autonomic dysfunction is unlikely to be secondary to medication. Longitudinal studies of autonomic symptoms are needed to more precisely delineate the temporal association between depression to autonomic dysfunction in HD.

There are no reasons to suspect damage to the peripheral nervous system in HD,<sup>5-7</sup> so the site of autonomic dysfunction is more likely found in the central autonomic network including the hypothalamus and its connections to the cortex, limbic system, brainstem and spinal cord.<sup>24</sup> Substantial hypothalamic pathology has indeed been found in both HD patients and animal models of the disease.<sup>3,25</sup> Recently, a significant loss of dopamine D<sub>2</sub> receptors as well as microglia activation was found in the hypothalamus of both HD patients and premanifest mutation carriers.<sup>26</sup> As these abnormalities as well as neuronal inclusions of mutant huntingtin were seen throughout the hypothalamus,<sup>26,27</sup> hypothalamic damage might account for the autonomic dysfunction that encompasses both sympathetic and parasympathetic functions.<sup>4-7</sup> Further studies combining autonomic functional tests with whole brain, and in particular hypothalamic, assessment are needed to more accurately pinpoint the sites of pathology within the central autonomic network in HD.

In conclusion, autonomic symptoms are highly prevalent in HD patients, may even precede the onset of motor signs, and are related to functional disability and depression.

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## PART II

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# HYPOTHALAMIC PATHOLOGY IN HUNTINGTON'S DISEASE





*“Here in this well-concealed spot [referring to the hypothalamus], almost to be covered with a thumbnail, lies the very main spring of primitive existence – vegetative, emotional, reproductive – on which with more or less success, man has come to superimpose a cortex of inhibitions.”*

**Harvey Cushing** *Papers relating to the Pituitary Body, Hypothalamus, and Parasympathetic Nervous System.* Charles C. Thomas, Springfield and Baltimore, 1932.



### **Hypocretin and melanin-concentrating hormone in patients with Huntington's disease**

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## ABSTRACT

To evaluate whether hypocretin-1 (orexin-A) and melanin-concentrating hormone (MCH) neurotransmission are affected in patients with Huntington's disease (HD), we immunohistochemically stained hypocretin and MCH neurons and estimated their total numbers in the lateral hypothalamus of both HD patients and matched controls. In addition, hypocretin-1 levels were determined in prefrontal cortical tissue and post-mortem ventricular cerebrospinal fluid (CSF) using a radioimmunoassay. The total number of hypocretin-1 neurons was significantly reduced by 30% in HD brains ( $p=0.015$ ), while the total number of MCH neurons was not significantly altered ( $p=0.100$ ). Levels of hypocretin-1 were 33% lower in the prefrontal cortex of HD patients ( $p=0.025$ ), but ventricular CSF levels were similar to control values ( $p=0.306$ ). Neuronal intranuclear and cytoplasmic inclusions of mutant huntingtin were present in all HD hypothalami, although with a variable distribution across different hypothalamic structures. We found a specific reduction in hypocretin signalling in patients with HD as MCH cell number was not significantly affected. It remains to be shown whether the moderate decrease in hypocretin neurotransmission could contribute to clinical symptoms. As the number of MCH expressing neurons was not affected, alterations in MCH signalling are unlikely to have clinical effects in HD patients.

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder caused by an expanded CAG trinucleotide repeat in the *IT15* gene on chromosome 4. Choreiform movements, psychiatric and behavioural problems and cognitive impairment characterize HD.<sup>16</sup> Other debilitating but less well-known features of the disease are weight loss, sleep disturbances and autonomic nervous system dysfunction,<sup>2</sup> the causes of which are poorly understood. HD is neuropathologically characterized by generalized atrophy and cell death in the striatum and cerebral cortex and the presence of neuronal intranuclear and cytoplasmic inclusions of mutant huntingtin, particularly in the neocortex and neostriatum.<sup>8,41</sup> Substantial hypothalamic atrophy and cell death have also been reported,<sup>18,20,21</sup> however, the presence of HD inclusions has not been investigated so far in different hypothalamic structures.

Hypocretin/orexin and melanin-concentrating hormone (MCH) are neuropeptides that are synthesized in the lateral hypothalamus by two distinct neuronal populations.<sup>30,31,36</sup> These neuropeptides both play a key role in the regulation of body energy metabolism, sleep-wake cycles and autonomic functions.<sup>17,30,33,43</sup> Moreover, recent animal studies implicate the MCH system in the modulation of several behavioural modalities, most notably stress, depression and sexual behaviour.<sup>30,34</sup> A relation has also been demonstrated between hypocretin release and some psychiatric symptoms.<sup>5,32</sup> Potential alterations in hypocretin and/or MCH signalling might thus contribute to some symptoms in HD patients, particularly weight loss, sleep disturbances and autonomic dysfunction as well as some behavioural disorders like increased rates of depression and anxiety in these subjects.<sup>16</sup>

Recently, it was demonstrated that the R6/2 mouse, the most widely used model of HD that expresses the first exon of the *HD* gene with ~150 CAG repeats, exhibits a progressive and massive loss of hypocretin-1 immunopositive neurons in the lateral hypothalamic area.<sup>27</sup> This loss amounted to 71% at the end stage (12 weeks) and was accompanied by loss of neuronal nuclear antigen (NeuN)-immunopositive neurons.<sup>27</sup> On the other hand, the YAC128 mouse model of HD with the full-length mutant *HD* gene with ~120 CAG repeats, shows a 10% loss of hypocretin-1 immunopositive neurons at 12 months.<sup>4</sup> Furthermore, MCH cell number was reported to be decreased by 38% in the hypothalamus of twelve week old R6/2 mice, while MCH peptide levels were reduced by 57%.<sup>39</sup> Atrophy and a decreased density of hypocretin-1 expressing neurons in single coronal sections from the lateral hypothalamus have also been observed in HD patients.<sup>27</sup> Nevertheless, four recent papers reported normal hypocretin-1 concentrations in the cerebrospinal fluid (CSF) of HD patients.<sup>3,4,11,24</sup>

In order to validate and extend the above findings in patients with HD we applied a four-way approach. First, we estimated the total numbers of both hypocretin-1 and MCH expressing neurons in the lateral hypothalamus of HD patients and matched controls. This allowed testing for the specificity of potential changes in the neuronal numbers. Second, we measured hypocretin-1 levels in post-mortem ventricular CSF because this could better reflect hypocretin-1 production than spinal measurements.<sup>7,10</sup> Third, hypocretin-1 contents in peptide extracts from cerebral cortex were assessed, since this has been shown to be a more sensitive technique compared to CSF measurements.<sup>10,28</sup> And finally, we also investigated various hypothalamic regions, including the lateral hypothalamus, for the presence of neuronal intranuclear and cytoplasmic inclusions.

## MATERIALS AND METHODS

### *Post-mortem material*

Autopsy hypothalami from eight HD patients and eight controls (matched for age, sex, post-mortem delay and fixation time) were obtained through the Netherlands Brain Bank (NBB). Ventricular CSF was available in 7 of these HD patients and one of the controls; therefore CSF from six additional controls (matched for age, sex and post-mortem delay) was used for comparison (Table 1). Frozen prefrontal cortical tissue from a second group of 19 HD patients and 16 controls was obtained through the Leiden University Medical Centre HD pathology archives (Table 2). HD brains were graded according to the scheme of Vonsattel et al. for neuropathological disease severity.<sup>41</sup> All HD patients had clinical features and a positive family history of the disease; one patient (#1; Table 2) had infantile HD and was studied separately as there are indications that infantile and classical HD may differ neuropathologically.<sup>35</sup> The diagnosis of HD was genetically confirmed (i.e. CAG repeat lengths  $\geq 40$ ) in all but one of these patients (#HD-8, Table 1). However, the latter patient's brain showed HD pathology grade II and the presence of neuronal intranuclear and cytoplasmic inclusions combined with a positive family history of the disease further consolidated the diagnosis. Exclusion criteria for control subjects were primary neurological and/or psychiatric disorders and glucocorticoid therapy during the final premortal illness period, except two controls who had suffered strokes and were used for prefrontal cortex hypocretin-1 measurements (#C-4 and #C-14; Table 2).

### *Hypocretin-1 and MCH immunohistochemistry*

The hypothalami were fixed in 10% PBS (pH 7.4) formalin at room temperature and were paraffin embedded and serially sectioned at 6  $\mu\text{m}$  in rostral-caudal direction. Every 100<sup>th</sup> section was stained with thionin for orientation. The lateral hypothalamus, from the level where the fornix abuts the paraventricular nucleus up to the posterior border of the corpora mamillaria, was stained at 600  $\mu\text{m}$  intervals in every two consecutive sections. One of the sections was stained with a hypocretin-1 and the other with a MCH monoclonal antibody (Phoenix Pharmaceuticals, Inc., Belmont, CA; catalog no. H-003-30 and H-070-47, respectively). The sections were visualized according to the avidin-biotin complex method using diaminobenzidine-nickel solution to finish the staining as described previously.<sup>13</sup>

### *N-terminal huntingtin immunohistochemistry*

Using thionin staining for orientation, nine to ten sections were chosen so that large parts of the following hypothalamic (and adjacent) structures would be contained in at least two coronal cuts: the suprachiasmatic nucleus, the supraoptic nucleus, the paraventricular nucleus, the infundibular nucleus, the diagonal band of Broca, the nucleus basalis of Meynert, the ventromedial nucleus, the dorsomedial nucleus, the lateral hypothalamus/perifornical area, the tuberomammillary nucleus, the lateral tuberal nucleus and the supramammillary area (the supramammillary area was contained in only one coronal cut). These sections were stained with a monoclonal antibody against the N-terminus of human huntingtin (Chemicon, Temecula, CA; batch no. 5374) after pretreatment by boiling in citrate buffer (pH 6.0) for 20 minutes. The sections were processed according to the avidin-biotin complex method using diaminobenzidine-nickel solution and counterstained with Harris's Hematoxylin for nuclear staining.

### *Antibody specificity*

The specificity of the hypocretin-1 antibody has been confirmed previously.<sup>9</sup> To test the specificity of



**Table 1. Clinicopathological details of HD patients and control subjects.**

		Sex	Age (yrs)	Age of onset (yrs)	PMD (h)	Fix (d)	Brain weight (g)	Grade	CAG repeat length	Cause of death	MCH cell no.	Hcrt-1 cell no.	Hcrt-1 levels in CSF (pg/ ml)
<b>Patients</b>	HD-1	M	57	42	07:30	53	1162	3-4	46	Cachexia	76987	42088	536
	HD-2	F	50	35	05:40	55	1292	2-3	47	Pneumonia	N.A.	24022	595
	HD-3	M	79	54	06:15	34	1001	4	44	Pneumonia and sepsis	75652	29890	422
	HD-4	F	67	56	06:05	41	1289	1	45	Unknown	102436	50863	531
	HD-5	M	49	40	05:45	49	1122	3	54	Cachexia secondary to pneumonia.	N.A.	20448	468
	HD-6	F	80	58	07:15	49	906	2	41	Pneumonia	76620	36023	404
	HD-7	M	61	39	10:25	48	1380	3	43	Pneumonia	77846	27880	481
	HD-8	M	54	41	03:50	80	1212	2	-	Sudden death	N.A.	42569	N.A.
<b>Median</b>			59.0	41.5	6:10	49.0	1187.0	-	45.0		76987	32957	
<b>Percentile</b>													
<b>25<sup>th</sup>:</b>			51.0	39.3	5:41	42.8	1031.3		43.0		76136	24987	
<b>75<sup>th</sup>:</b>			76.0	55.5	7:26	54.5	1291.3		47.0		90141	42449	
<b>Controls</b>	C-1 <sup>a</sup>	M	58	-	<17:00	96	1408	-	-	Aorta dissection	77237	49105	N.A.
	C-2 <sup>a</sup>	F	49	-	<13:30	165	1437	-	-	Metastasized cervix carcinoma.	89347	56250	N.A.
	C-3 <sup>a</sup>	M	79	-	<3:00	53	1435	-	-	Haemorrhage from leaking aorta prosthesis	84652	36231	N.A.
	C-4 <sup>a</sup>	F	68	-	05:45	32	1153	-	-	Unknown	N.A.	52900	N.A.
	C-5 <sup>a</sup>	M	49	-	<12:40	40	1404	-	-	Sudden death	118604	54638	N.A.
	C-6 <sup>a,b</sup>	F	82	-	05:30	36	1280	-	-	Myocardial infarction	N.A.	60972	409
	C-7 <sup>a</sup>	M	61	-	13:50	52	2220	-	-	Carcinoma of the oesophagus	94776	27912	N.A.
	C-8 <sup>a</sup>	M	54	-	<08:00	59	1350	-	-	Hepatocellular carcinoma	86792	53216	N.A.
	C-9 <sup>b</sup>	M	56	-	5:25	-	1522	-	-	Cardiac infarction	N.A.	N.A.	536
	C-10 <sup>b</sup>	F	51	-	7:40	-	1156	-	-	Sepsis	N.A.	N.A.	640
	C-11 <sup>b</sup>	M	79	-	6:00	-	1392	-	-	Mestastasized adenocarcinoma	N.A.	N.A.	631
	C-12 <sup>b</sup>	F	69	-	4:20	-	1186	-	-	Heart failure	N.A.	N.A.	549
	C-13 <sup>b</sup>	M	53	-	14:25	-	1341	-	-	Heart failure	N.A.	N.A.	581
	C-14 <sup>b</sup>	M	61	-	12:05	-	1460	-	-	Heart failure	N.A.	N.A.	336
<b>Group a:</b>													
<b>Median</b>			59.5		10:20	52.5	1406.0				88070	53058	-
<b>Percentiles</b>													
<b>25<sup>th</sup>:</b>			50.3		5:33	37.0	1297.5				82798	39450	
<b>75<sup>th</sup>:</b>			76.3		13:45	86.8	1436.5				100733	55847	
<b>Group b:</b>													
<b>Median</b>			61.0		6:00	-	1341.0				-	-	549.0
<b>Percentiles</b>													
<b>25<sup>th</sup>:</b>			53.0		5:25		1186.0						409.0
<b>75<sup>th</sup>:</b>			79.0		12:05		1460.0						631.0

<sup>a</sup>) Hypothalamic material from these controls was available for Hcrt-1 and/or MCH immunohistochemistry.

<sup>b</sup>) Ventricular CSF from these controls was available for Hcrt-1 radioimmunoassay.

**Legend:** Fix = fixation time, PMD = post-mortem delay, Grade = Vonsattel et al's grade, N.A. = not available. Hcrt-1, hypocretin-1.

**Table 2. Clinicopathological data of subjects used for hypocretin brain tissue measurement.**

	subject no.	Age at death	Sex	Grade	CAG	Cause of death	Hcrt-1 tissue content (pg/g)
<b>Infantile HD</b>	1 <sup>a</sup>	19	F	3	86	Cachexia	1097
<b>Adult HD</b>	2	34	M	3	52	Unknown	389
	3	40	F	3	41	Suicide	530
	4	44	M	N.A.	50	Pneumonia	343
	5	45	M	4	53	Recurrent aspiration pneumonia	198
	6	49	M	3	47	Respiratory insufficiency	743
	7	51	M	3	46	Bronchopneumonia	360
	8	51	M	3	45	Unknown	584
	9	53	F	2	47	Pulmonary embolism	511
	10	54	M	3	43	Pneumonia	198
	11	55	F	3	47	Metast. Grawitz tumor	617
	12	57	F	3	43	Pulmonary embolism	278
	13	57	M	4	49	Bronchopneumonia	275
	14	57	M	3	47	Unknown	665
	15	63	F	3	43	Basilar artery thrombosis	1066
	16	66	F	3	41	Squamous cell lung carcinoma	443
	17	69	F	2	42	Aspiration pneumonia	725
	18	75	M	3	43	Unknown	302
	19	77	M	3	39	Bronchopneumonia	376
<b>Median</b>		54.5		3.0	45.5		416.4
<b>Percentiles (25<sup>th</sup> – 75<sup>th</sup>)</b>		48.0 – 63.8		2.5 – 3.0	42.8 – 47.5		295.8 – 629.4
<b>Controls</b>	1	37	M	-	-	Myocardial infarction	996
	2	46	M	-	-	Cardiac arrhythmia	364
	3	48	M	-	-	Myocardial infarction	665
	4	52	M	-	-	Stroke	688
	5	60	F	-	-	Pneumonia	433
	6	63	F	-	-	Leptomeningeal metastasis	737
	7	64	M	-	-	Myocardial infarction	1368
	8	67	F	-	-	Aortic Dissection	858
	9	68	M	-	-	Pneumonia	697
	10	70	M	-	-	Metastasized carcinoma	1282
	11	71	M	-	-	Cardiomyopathy	573
	12	74	M	-	-	Metastasized carcinoma	243
	13	76	M	-	-	Aortic Dissection	638
	14	79	F	-	-	Stroke	892
	15	83	M	-	-	Leptomeningeal metastasis	358
	16	86	M	-	-	Myocardial infarction	570
<b>Median</b>		67.5					676.6
<b>Percentiles (25<sup>th</sup> – 75<sup>th</sup>)</b>		54.0 – 75.5					467.5–883.9

<sup>a</sup>) This patient had juvenile HD and was therefore studied separately.

**Legend:** Grade = Vonsattel et al's grade, CAG = CAG repeat length, Hcrt-1, hypocretin-1, N.A. = not available.

the MCH antibody, a dot blot was performed,<sup>40</sup> adding a dilution of 1:1000 anti-MCH onto 2% gelatin-coated nitrocellulose paper (0.1- $\mu$ m pore size) containing different spots with 20 pmol MCH, hypocretin-1, somatostatin (1–14), somatostatin (1–28), galanin, MCH-1 receptor,  $\beta$ -lipotropin, substance-P,  $\alpha$ -melanocyte-stimulating hormone, luteinizing hormone-releasing hormone, adrenocorticotrophic hormone (1–39), neurotensin, oxytocin, corticotropin-releasing hormone, agouti-related protein (83–132), neuropeptide-Y, growth hormone-releasing hormone (1–40), arginine-vasopressin, desacetylmelanocyte-stimulating hormone, neuropeptide EI, glycoprotein hormone receptor and cocaine- and amphetamine-regulated transcript. The next day, the nitrocellulose sheet was incubated with secondary antibody, avidin-biotin peroxidase complex, and diaminobenzidinenickel solution to finish the staining. The only spot that showed staining was the one containing MCH. Specificity was further confirmed by the absence of staining in hypothalamic sections using antiserum preadsorbed with the human MCH peptide fixed overnight with 4% formaldehyde onto gelatin-coated nitrocellulose filter paper, 0.1  $\mu$ m, and the presence of staining when preadsorbed with  $\alpha$ -melanocyte-stimulating hormone peptide, which did not differ from unadsorbed serum.

### ***Quantification of hypocretin-1 and MCH neuronal numbers***

An estimate of the total number of hypocretin-1 and MCH immunoreactive (IR) cells was made using an image analysis system (ImagePro version 4.5, Media Cybernetics, Silver Spring) connected to a camera (JVC KY-F553CCD) and plain objective microscope (Zeiss Axioskop with Plan-NEOFLUAR Zeiss objectives, Carl Zeiss GmbH, Jena, Germany). In every section randomly selected fields, covering in total 15% of a manually outlined area containing all the hypocretin-1 or MCH IR cells, were counted by the same person (NAA) who was blinded to the diagnosis. Each positively stained profile containing a nucleolus was counted.<sup>10</sup> Calculation of the total number of hypocretin-1 IR neurons was performed by a conversion program based upon multiplication of the neuronal counts by sample frequency of the sections as described previously.<sup>14</sup> Mean ( $\pm$  SD) number of sections quantified per subject was  $10.2 \pm 1.5$  for hypocretin-1 and  $11.5 \pm 2.3$  for MCH. The coefficient of variation (i.e. SD / mean  $\times$  100%) of this method was 3.4% for hypocretin-1 and 4.5 % for MCH (calculated by counting one complete control twice).

### ***Hypocretin-1 measurements in cerebral cortex and CSF***

One gram of frozen prefrontal cortex was used for hypocretin-1 measurements (Table 2). The most rostral part of the prefrontal cortex was chosen, as this cortical region is densely innervated by hypocretin neurons resulting in high hypocretin-1 concentrations.<sup>29</sup> The tissue samples were boiled for 10 minutes in 10.0 ml of MilliQ water, cooled to room temperature, acidified using glacial acetic acid and HCl (final concentration: 1.0 M and 20.0 mM respectively), homogenized and centrifuged. The supernatant was acidified again with an equal volume of 0.1% trifluoroacetic acid and vacuum dried. Samples were resuspended in 500  $\mu$ l of radioimmunoassay buffer and centrifuged at 3000 rpm for 10 minutes before measurements. Immediately after collection, ventricular CSF was centrifuged at 2500 rpm for 10 minutes and the supernatant was stored at -80°C until hypocretin-1 levels were measured using a commercially available radioimmunoassay (Phoenix Pharmaceuticals, Belmont, USA). All measurements were conducted in duplicate 100  $\mu$ l aliquots in a single assay run. The detection limit was 50 pg/ml and intra-assay variability was less than 5%. We used a validated reference sample to adjust levels to previously reported values.<sup>10,28</sup>

### ***Assessment of neuronal intranuclear and cytoplasmic inclusions***

The presence or absence of neuronal intranuclear inclusions and cytoplasmic inclusions (i.e. inclusions in dystrophic neurites) in the hypothalamic regions of interest were assessed by one of us (NAA) at  $\times 100$  magnification.<sup>8</sup> For each region two coronal sections were investigated. The percentage of HD patients who had either neuronal intranuclear or cytoplasmic inclusions of mutant huntingtin was calculated per structure (Table 4).

### **Statistics**

All data are presented as medians (25<sup>th</sup> – 75<sup>th</sup> percentile). Differences between the groups were statistically evaluated by the Mann-Whitney *U* (MWU) test, the Wilcoxon signed rank (WSR) test or the  $\chi^2$ -test. Spearman's  $\rho$  correlation test was performed to assess all correlations. Differences in clock time of death (circular parameter) between controls and patients with HD were tested with the Watson's two-sample test of homogeneity.<sup>23</sup> Tests were two-tailed and values of  $p < 0.05$  were considered to be significant. Local linear regression was used to fit curves to the standardized pooled data (Figure 2).<sup>22</sup>

## **RESULTS**

### ***Hypocretin-1 and MCH neurons in HD patients and controls***

#### *Subject characteristics*

There were no significant differences in age, sex, post-mortem delay, fixation time and clock time of death between the HD and the control group (all  $p \geq 0.27$  (Table 1)).

#### *Distribution of hypocretin-1- and MCH neurons*

The intensity of hypocretin-1 and MCH immunostaining showed no obvious differences between the HD and the control group (Figure 1). Hypocretin-1 IR neurons were restricted to the perifornical region in the lateral hypothalamus as previously described.<sup>9</sup> MCH IR neurons were mainly confined to the same areas although they were more widely disseminated than hypocretin-1 IR neurons. The first hypocretin-1 IR neurons emerged in the lateral hypothalamus at the junction of the fornix and the paraventricular nucleus and were followed by the first MCH IR neurons. However, the rostro-caudal distance between the location of the first hypocretin-1 neurons and that of the first MCH neurons did not significantly differ from zero (either in patients ( $n = 8$ ) or controls ( $n = 8$ ) or both groups combined (respective  $p$ -values by WSR test: 0.168, 0.336 and 0.085)) (Figure 2). At subsequent levels the fornix was surrounded by both hypocretin-1 and MCH IR cell bodies throughout its entire course up to the mamillary bodies. The number of hypocretin-1 IR neurons peaked just before the fornicomamillary junction, whereas the peak number of MCH IR neurons was seen after this junction in the supramamillary area (Figure 2); the rostro-caudal distance between the two peaks was calculated in each individual and was significantly larger than zero (all  $p \leq 0.011$  by WSR test, either in patients (1200  $\mu\text{m}$  [1200-1800]) or controls (1500  $\mu\text{m}$  [1200-2250]), or both groups combined (1200  $\mu\text{m}$  [1200-1800])).

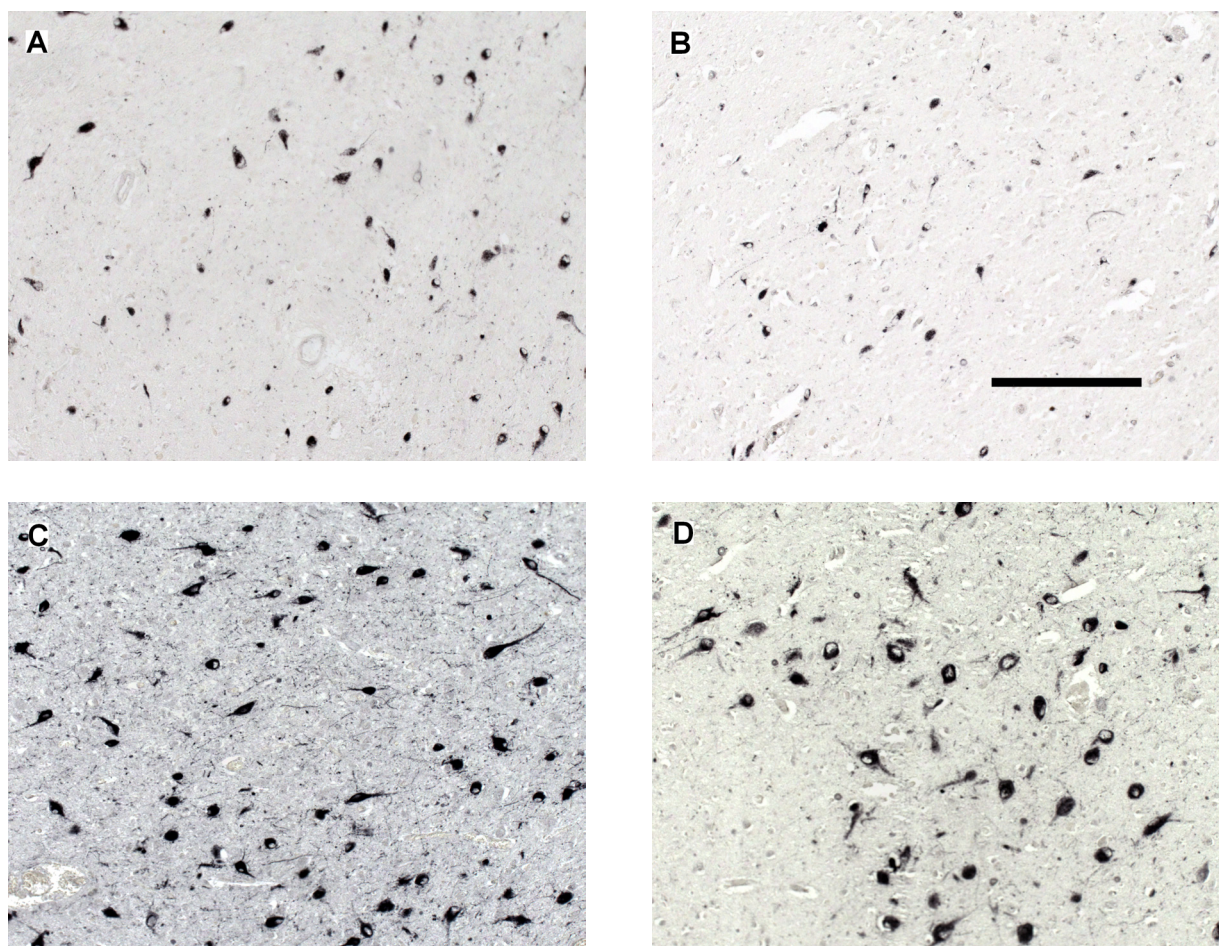
#### *Hypocretin-1 and MCH cell numbers*

The total number of hypocretin-1 IR neurons in the lateral hypothalamus of HD patients was significantly reduced by 30 % compared to values in matched controls (32,957 (24,987 – 42,449) vs. 53,058 (39,450 –



55,847);  $p = 0.015$ ; Figure 3). As the available hypothalamic material of two controls and three HD patient (#C-4, #C-6 and #HD-2, #HD-5 and #HD-8, respectively (Table 1)) did not contain the caudal part of the MCH area, these cases were excluded from subsequent calculations of the total number of MCH IR neurons. Exclusion of these subjects did not alter group comparability. There was a trend towards a decrease in the total number of MCH IR neurons in HD patients (HD: 76,987 (76,136 – 90,141); controls: 88,070 (82,798 – 100,733);  $p = 0.100$ ; Figure 3).

**Figure 1.** Representative photographs of hypocretin-1 and MCH IR neurons in the lateral hypothalamus of two control subjects (A and C, #C-4 and #C-2 respectively) and two patients with HD (B and D, #HD-6 and #HD-1 respectively) with neuronal counts at or around the median (Table 1). The pictures were taken from slides with the highest numbers of immunoreactive neurons. The illustrations show a modest reduction in the number of hypocretin-1 IR neurons in the HD brain (A and B) while the number of MCH IR neurons is not significantly affected (C and D). Scale bar 250  $\mu$ m.

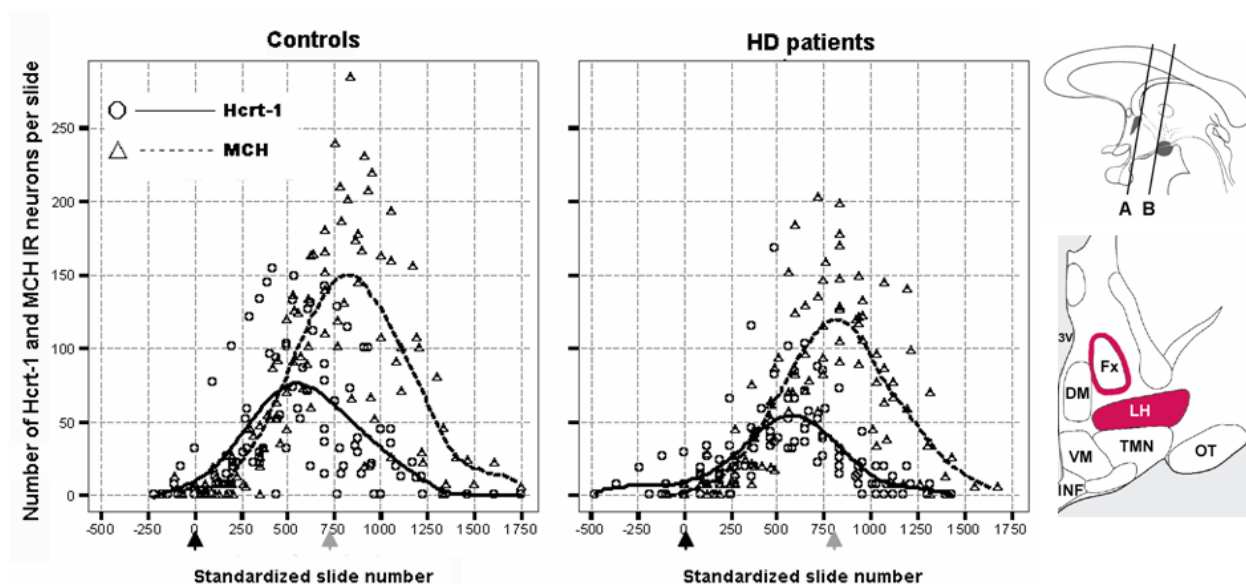


*Effects of age of onset, CAG repeat length, disease duration and Vonsattel et al's grade on hypocretin-1 and MCH cell numbers in HD*

There was a positive trend for the relation between age of onset of HD (defined as the age at which the clinical diagnosis was first made) and hypocretin-1 IR neuronal numbers ( $r = 0.64$ ,  $p = 0.086$ ), but not MCH neuronal numbers ( $r = 0.21$ ,  $p = 0.645$ ). In HD patients, the total hypocretin-1 and MCH cell numbers were

not significantly associated with either CAG repeat length, duration of illness or Vonsattel et al's grades of neuropathological disease severity (Table 3).<sup>41</sup>

**Figure 2.** Distribution patterns of hypocretin-1 and MCH IR neurons in the lateral hypothalamus of controls and HD patients. In order to present data from all subjects in the same diagrams, the individual distribution patterns were standardized in rostro-caudal direction for the anatomical distance between the point where the fornix abuts the paraventricular nucleus (black arrows; at the level of line 'A' in the upper cartoon) and the fornicomamillary junction (grey arrows; at the level of line 'B' in the upper cartoon). This procedure was performed separately for the control and the HD group. In addition, local linear regression was used to fit curves to the standardized pooled data in order to clarify the underlying distribution patterns (kernel = Gaussian, bandwidth value = 1.0). Note that the overall rostro-caudal dispersion of hypocretin-1 and MCH IR neurons does not appear to be noticeably different between control and HD subjects. Upper cartoon: A schematic sagittal view of the hypothalamus; the paraventricular nucleus and the mamillary bodies are indicated in dark. Lower cartoon: A schematic coronal view of the hypothalamus midway between lines 'A' and 'B' in the upper cartoon; the perifornical area and the lateral hypothalamus are indicated in dark (adapted from (36)). Hcrt-1, hypocretin-1; 3V, third ventricle; Fx, fornix; LH, lateral hypothalamus; DM, dorsomedial nucleus; VM, ventromedial nucleus; TMN, tuberomamillary nucleus; INF, infundibular nucleus; OT, optic tract.



### Ventricular CSF hypocretin-1 content

The two groups were well matched for age, sex and post-mortem delay (all  $p > 0.80$ ) (Table 2). Furthermore, there were no significant correlations between these variables and hypocretin-1 CSF contents in HD patients, controls or the combined group (all  $p > 0.11$ ). The CSF contents of hypocretin-1 was not different between HD patients and controls (HD: 481 pg/ml (422 – 536); controls: 549 pg/ml (409 – 631);  $p = 0.306$ , Figure 4), nor was there a significant correlation between the hypocretin cell counts and CSF levels in the 8 subjects (7 HD patients and 1 control) in whom both measurements were available ( $r = -0.238$ ;  $p = 0.570$ ). There were

no significant correlations between CSF hypocretin-1 levels and other (clinical) disease parameters (Table 3).

**Table 3. Spearman's  $\rho$  correlations (p-values) between post-mortem findings and other (clinical) disease parameters in HD patients.** The only significant correlation was between the prefrontal cortex Hcrt-1 levels and Vonsattel et al.'s grade of neuropathological disease severity. Trends were visible for the relations between Hcrt-1 cell number and age of onset and between MCH cell number and disease duration.

	Age at death	Age of onset	Disease duration	Grade	CAG repeat no.
Hcrt-1 cell no.	0.452 (0.260)	0.643 (0.086)	-0.096 (0.820)	-0.482 (0.227)	-0.429 (0.337)
MCH cell no.	-0.500 (0.391)	-0.200 (0.747)	-0.821 (0.089)	-0.700 (0.188)	0.300 (0.624)
Ventricular CSF Hcrt-1 levels	-0.607 (0.148)	-0.607 (0.148)	-0.436 (0.328)	-0.090 (0.848)	0.571 (0.180)
Prefrontal cortex Hcrt-1 levels	0.078 (0.760)	-0.417 (0.265)	0.192 (0.620)	-0.666 (0.004)*	-0.179 (0.476)

\* Correlation is significant at the 0.01 level.

### *Hypocretin-1 concentration in prefrontal cortex*

The two groups did not differ with respect to gender, but the HD group was significantly younger ( $p = 0.034$ ) (Table 2). However, hypocretin-1 concentrations were not significantly correlated with age (either in HD patients, controls, or the combined groups (all  $p \geq 0.37$ )). Hypocretin-1 concentration in controls was 676.6 (467.5 – 883.9) pg/gram of wet brain tissue, which is comparable to previously reported values (28). Compared to controls mean hypocretin-1 cortical levels were 33% lower in adult HD patients (416.4 pg/g (295.8 – 629.4);  $p = 0.025$ ). Furthermore, the prefrontal hypocretin-1 levels in these patients were significantly associated with the Vonsattel et al's grades ( $r = -0.666$ ,  $p = 0.004$ ), but not with other (clinical) disease parameters (Table 3). The patient with infantile HD had the highest levels of cortical hypocretin among all the HD patients studied (Table 2). Since the prefrontal tissues belonged to subjects whose hypocretin cell counts were unavailable, we could not relate the prefrontal cortex levels of hypocretin-1 to the hypocretin cell counts.

### *Neuronal intranuclear and cytoplasmic inclusions in HD hypothalami*

N-terminal huntingtin-positive neuronal intranuclear and cytoplasmic inclusions were observed in all HD hypothalami (Figure 5). Cytoplasmic inclusions were far more abundant than intranuclear inclusions which were seen only sporadically. Intranuclear inclusions were most consistently observed in the neurons of the tuberomammillary nucleus. The HD inclusions were not uniformly distributed among the various hypothalamic and adjacent structures (Table 4). Only in relatively few patients cytoplasmic inclusions were present in the most rostral hypothalamic structures (i.e. the suprachiasmatic, the supraoptic, and the paraventricular nuclei), while intranuclear inclusions could not be detected at all in these areas.

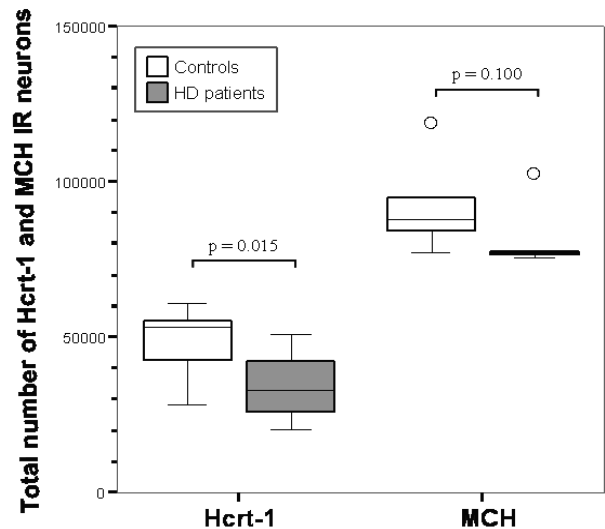


**Table 4. Neuronal inclusions in nuclei of HD hypothalami and adjacent regions.**

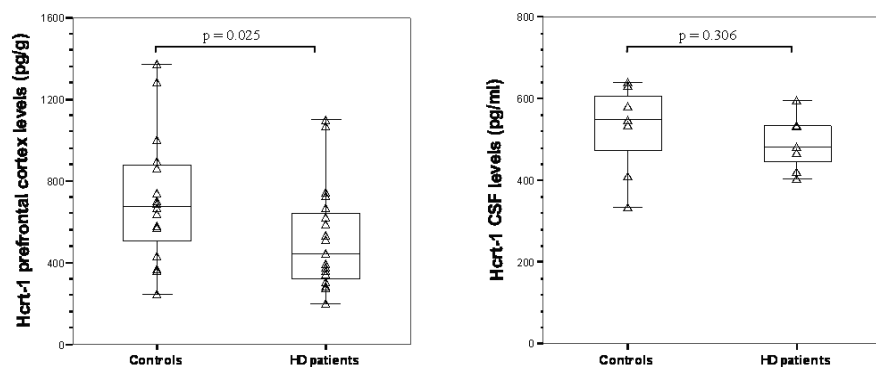
Type	SCN		SON		PVN		DBB/ NBM		INF		VMN		DMN		LH/ PFA		NTL		TMN		SMA	
	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
HD-1	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	+	+	-
HD-2	-	-	-	-	-	-	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	-
HD-3	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-
HD-4	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-	+	+	+	-
HD-5	+	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-
HD-6	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-
HD-7	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-
HD-8	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-
% patients	50	0	50	0	63	0	100	25	100	25	100	13	100	0	100	25	75	13	100	75	100	0

Legend: Type = type of neuronal inclusions; C = cytoplasmic inclusions (i.e. inclusions in dystrophic neurites); I = intranuclear inclusions; - = inclusions absent; + = inclusions present. The last row indicates, per structure, the percentage of HD patients who had neuronal inclusions of the specified type. SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; PVN, paraventricular nucleus; INF, infundibular nucleus; DBB, diagonal band of Broca; NBM, nucleus basalis of Meynert; VMN, ventromedial nucleus; DMN, dorsomedial nucleus; LH, lateral hypothalamus; PFA, perifornical area; NTL, lateral tuberal nucleus; TMN, tuberomamillary nucleus; SMA, supramamillary area.

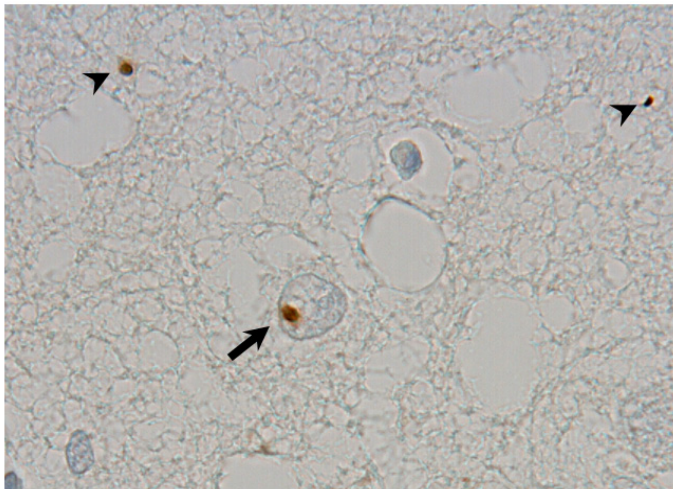
**Figure 3.** The total numbers of hypocretin-1 and MCH IR neurons in the lateral hypothalamus of control subjects and HD patients. The total number of hypocretin-1 IR neurons is significantly decreased in HD, while there is only a trend towards a reduction in the total number of MCH IR neurons (MWU-test:  $n = 16$ ,  $p = 0.015$  for Hcrt-1 and  $n = 11$ ,  $p = 0.100$  for MCH). Outliers (defined as data points which lie 1.5 times the interquartile range below the first or above the third quartile) are symbolized by 'o'. Hcrt-1, hypocretin-1.



**Figure 4.** Compared to controls, hypocretin-1 levels were significantly lower in the prefrontal cortex of HD patients (MWU-test:  $p = 0.025$ ), but not in their CSF (MWU-test:  $p = 0.306$ ). Hcrt-1, hypocretin-1.



**Figure 5.** Examples of neuronal intranuclear (arrow) and cytoplasmic (arrowheads) inclusions of mutant huntingtin in the tuberomammillary nucleus of one HD patient (#HD-7; obj.  $\times 60$  oil).



## DISCUSSION

In this study, we demonstrate a significant reduction by about 30% in the total number of hypocretin-1 neurons in the lateral hypothalamus of HD patients. This decrease appears to be relatively specific as the total number of MCH neurons was not significantly altered. Hypocretin-1 levels in the prefrontal cortex were reduced to the same extent, but ventricular CSF levels were unchanged. Furthermore, we describe the distribution of intranuclear and cytoplasmic inclusions of mutant huntingtin in the lateral hypothalamus and various other hypothalamic and adjacent structures in patients with HD.

Recently the density of hypocretin-1 neurons was assessed in single coronal sections from the lateral hypothalamus of HD patients and found to be decreased.<sup>27</sup> However, cell density is subject to substantial variation depending on both the rostro-caudal level of the sections (Figure 2) and the cutting direction. Therefore, in this study we systematically counted the *total* number of hypocretin-1 expressing neurons and were able to unequivocally confirm a significant but moderate decrease in the number of these neurons in HD patients. A positive trend was also visible for the relation between hypocretin-1 cell number and age of onset in HD patients paralleling findings by Kremer et al.<sup>21</sup> who found an association between neuronal numbers in the lateral tuberal nucleus and age of onset in HD.

We corroborated the specificity of the reduction in hypocretin-1 neuronal numbers by assessing the total number of MCH neurons as well. Since the reduction in hypocretin-1 neuronal numbers was more pronounced than that in MCH neuronal numbers, hypocretin neurons appear to be more vulnerable to the pathogenic mechanisms underlying HD. Interestingly, a recent study in rat hypothalamic slice cultures showed that 24-hours of incubation with N-methyl-D-aspartate (NMDA) resulted in a marked decrease in the number of hypocretin-1 neurons, whereas MCH neurons in the same cultures were relatively spared.<sup>19</sup> Moreover, examinations of the effects of several endogenous glutamate receptor agonists highlighted quinolinic acid as an endogenous excitotoxin that could cause selective loss of hypocretin-1 neurons as compared to MCH neurons by activating NMDA receptors.<sup>19</sup> Therefore, NMDA receptor-mediated excitotoxicity could be involved in the greater susceptibility of hypocretin-1 neurons in HD patients, a pathomechanism that has also been proposed for the massive neuronal loss in the lateral tuberal nucleus of these patients.<sup>21</sup>

To further assess hypocretin neurotransmission in HD, we also examined hypocretin-1 contents in the prefrontal cortex and ventricular CSF. Whereas the mean levels of hypocretin-1 were about 30% lower in the

prefrontal cortex of HD subjects and correlated with Vonsattel et al's grades, ventricular CSF hypocretin-1 contents did not differ between patients and controls. The latter finding is in accordance with four recent papers that reported normal hypocretin-1 concentrations in the CSF of HD patients.<sup>3,4,11,24</sup> The apparent discrepancy between the findings in the CSF and those in the hypothalamus and the prefrontal cortex of HD patients could be accounted for by the fact that a mean reduction of approximately 30% in the number of hypocretin-1 neurons is probably not large enough to be reflected in the CSF.<sup>3,11,24</sup> This assumption is supported by the fact that a reduction by half in the number of hypocretin neurons in PD patients only causes a 25% decrease in hypocretin-1 levels in the ventricular CSF.<sup>10</sup> Accordingly, rat studies indicate that a 73% decline in hypocretin neuronal numbers is needed to decrease CSF hypocretin-1 levels by half.<sup>12</sup> Yet another possibility is impaired clearance of hypocretin-1 from the CSF in HD.

So far, hypocretin signalling has been studied in two animal models of HD. The R6/2 mouse model is reported to have a loss of more than 70% in both hypocretin-1 expressing neurons and hypocretin-1 CSF levels, whereas the YAC128 mouse model exhibits a reduction of 10% in the number of hypocretin-1 neurons. Moreover, R6/2 mice are reported to have a loss of almost 40% in the number of MCH expressing neurons while their hypothalamic MCH levels are reduced by nearly 60%.<sup>39</sup> The discrepancy between our findings and those from these transgenic mice could be accounted for by the existence of several confounding variables. First, these transgenic mouse models have very large CAG repeat expansions (> 120 repeats) and resemble juvenile HD more than the adult form of the disease.<sup>25</sup> An intriguing possibility is thus that juvenile HD patients might indeed exhibit more extensive pathology of the hypocretin system. In this study, we could measure hypocretin-1 levels in the prefrontal cortex of only one juvenile HD patient (Table 2). Surprisingly, this case appeared to have the highest levels of cortical hypocretin among all the HD patients studied. This finding may, however, be due to the stronger cortical atrophy that accompanies the juvenile variant of HD compared to the adult form of the disease.<sup>35</sup> Second, the mild reduction in hypocretin in the YAC128 mice may be due to the fact that these mice, unlike the R6/2 mice and several human patients, were not (close to) end stage at the time of assessment (i.e. 12 months). Thus, several variables may confound the comparisons between various animal models and the human condition and should, therefore, be taken into consideration when comparing animal and human data.

Hypocretin deficiency is the primary pathophysiological cause of narcolepsy, a sleep-wake disorder characterized by excessive daytime sleepiness and REM-sleep dissociation phenomena such as cataplexy, i.e. a sudden weakening of posture muscle tone usually triggered by emotion.<sup>26</sup> Although R6/2 mice exhibit episodes of behavioural arrest closely resembling those seen in *hypocretin* knock-out mice and transgenic mice with specific ablation of hypocretin-containing neurons,<sup>6,15</sup> it remains to be shown whether the modest decrease of hypocretin-1 signalling in adult HD patients could contribute to clinical symptoms, particularly sleep disturbances.<sup>42</sup> Unfortunately, due to the retrospective nature of our study we could not relate our post-mortem findings to clinical signs and symptoms. This stresses the need for systematic post-mortem brain tissue collection of clinically well-documented patients for future neuropathological studies.

In this report we also present an estimation of the total number of MCH neurons in the human brain and their

relative distribution with respect to hypocretin-1 neurons. Our results confirm those from a recent study<sup>38</sup> and suggest that MCH neurons are indeed more abundant in the human hypothalamus and have a wider rostro-caudal distribution than hypocretin-1 neurons, which is also in accordance with findings in rodents.<sup>1,37</sup> Even though MCH and hypocretin-1 neurons start to appear at about the same level rostrally, MCH neurons are relatively more abundant in the posterior hypothalamus. Whether this finding could be accounted for by the existence of several distinct MCH subpopulations along the rostro-caudal axis as opposed to a more homogenous hypocretin population<sup>1,37</sup> remains to be elucidated. As we could not find a clear reduction in the number of MCH neurons in HD patients, alterations in MCH levels are unlikely to have clinical implications in HD.

In this study we have assessed neuronal numbers by counting immunopositive neurons by means of a technique that has been validated and applied previously.<sup>9,10,38</sup> It should be stressed that it is in principle impossible to distinguish the loss of an immunocytochemical neuronal marker from the loss of the neurons in a heterogeneous and anatomically loosely defined brain structure such as the lateral hypothalamus.

Interestingly, neuronal intranuclear and cytoplasmic inclusions were not uniformly present in various hypothalamic and adjacent structures in HD patients. This finding may indicate that various hypothalamic nuclei are differentially affected by inclusion formation despite their close anatomical juxtaposition in the hypothalamus. Elucidation of the underlying mechanisms of this heterogeneity may lead to better understanding of why certain neuronal populations are more susceptible to HD pathology than others.

In conclusion, we found a specific reduction by about 30% in hypocretin signalling in patients with HD. It remains to be shown whether this moderate decrease in hypocretin signalling could contribute to clinical symptoms. As MCH cell number was not clearly affected in HD patients, alterations in MCH neurotransmission are unlikely to have clinical effects in HD.

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## PART III

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# **E**NDOCRINE STUDIES IN **H**UNTINGTON'S DISEASE





*“The functions of the supra-renal capsules ... are almost or altogether unknown. The large supply of blood which they receive from three separate sources; their numerous nerves, derived immediately from the semilunar ganglia and solar plexus; their early development in the foetus; their unimpaired integrity to the latest period of life; and their peculiar gland-like structure; all point to the performance of some important office.”*

**Thomas Addison** *On The Constitutional And Local Effects Of Disease Of The Supra-Renal Capsules*. Samuel Highley, London, 1855.

*“The facts which Addison has published seem to lead to the conclusion that these little organs are essential to life.”*

**Charles Edouard Brown-Séquard** *Recherches expérimentales sur la physiologie et al pathologie des capsules surrénales*. Archives Général de Médecine 1856(2); 385.



### **Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease**

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## ABSTRACT

*Context:* Huntington's disease (HD) is a fatal hereditary neurodegenerative disorder characterized by motor, cognitive and behavioral disturbances. Hypothalamic-pituitary-adrenal (HPA) axis dysfunction could contribute to a number of HD signs and symptoms, however, no data are available on cortisol diurnal variations and secretory dynamics in HD patients. *Objective:* To perform a detailed analysis of HPA axis function in HD patients in relation to clinical signs and symptoms. *Design, setting & participants:* Twenty-four hour cortisol secretion was studied in eight early-stage, medication-free HD patients and eight age-, sex- and body mass index (BMI)-matched controls in a clinical research laboratory. Cortisol levels were measured every 10-min. *Main outcome measures:* Multi-parameter auto-deconvolution and cosinor regression were applied to quantify basal, pulsatile and total cortisol secretion rates as well as diurnal variations in cortisol levels. *Results:* Total cortisol secretion rate and the amplitude of the diurnal cortisol profile were both significantly higher in HD patients compared with controls ( $3490 \pm 320$  vs.  $2500 \pm 220$  nmol/L/24h,  $p = 0.023$  and  $111 \pm 14$  vs.  $64 \pm 8$  nmol/L,  $p = 0.012$ , respectively). Cortisol concentrations in patients were particularly increased in the morning and early afternoon period. In HD patients, mean 24 h cortisol levels significantly correlated with total motor score, total functional capacity as well as BMI. *Conclusions:* HPA axis hyperactivity is an early feature of HD and is likely to result from a disturbed central glucocorticoid feedback due to hypothalamic pathology. HPA axis dysfunction may contribute to some signs and symptoms in HD patients.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat size in the gene encoding the protein huntingtin.<sup>1</sup> The disease is characterized by chorea, cognitive deterioration, and psychiatric and behavioral problems.<sup>1</sup> Other debilitating but less well-known features of HD are weight loss, sleep disturbances and autonomic nervous system dysfunction, the causes of which are poorly understood.<sup>2,3</sup> However, as neuronal inclusions of mutant huntingtin, the neuropathological hallmark of HD, and substantial atrophy and cell loss have been reported in the hypothalamus of HD patients, neuroendocrine perturbations may be involved.<sup>2,4-6</sup>

Recently, progressive alterations in the hypothalamic-pituitary-adrenal (HPA) axis function were reported in the R6/2 mouse, the most widely used transgenic model of HD.<sup>7</sup> R6/2 mice show progressive increases in serum and urine corticosterone levels which is accompanied by a Cushing-like syndrome.<sup>7</sup> Increased urine and serum cortisol levels have also been reported in HD patients.<sup>7-10</sup> However, these studies applied a single or a few baseline measurements, which is clearly not adequate to assess either the pulsatile nature of cortisol secretion or its robust diurnal rhythmicity. Apart from mean levels, both the pulsatile secretion patterns and the diurnal variations are thought to be essential for normal hormone function.<sup>11</sup> Indeed, substantial diurnal rhythm disturbances, attributed to aberrant signaling by the suprachiasmatic nucleus (SCN), have been described in both HD patients and animal models.<sup>12,13</sup> As the activity of the suprachiasmatic nucleus, the body's master clock, is accurately reflected in the diurnal fluctuations of plasma cortisol levels,<sup>14,15</sup> we hypothesized that both the diurnal rhythmicity of plasma cortisol and its secretion pattern are likely to be perturbed in HD patients.

We tested this hypothesis by applying circadian rhythmicity analysis and deconvolution of 24 h plasma cortisol concentration profiles in both early stage HD patients and healthy matched controls.

## SUBJECTS AND METHODS

### *Subjects*

Eight early-stage HD patients and eight 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 symptoms and signs.<sup>16</sup> All subjects were free of medication. 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. 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. Blood was allowed to clot and, within 60 min of sampling, all tubes were centrifuged at 4000 rotations/min at 4 °C for 20 min, and plasma was stored at -80 °C 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, catecholamines and cortisol 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***

Plasma cortisol was measured by radioimmunoassay (GammaCoat™, DiaSorin, Stillwater, Minnesota, USA). The detection limit of the assay was 25 nmol/L, and the interassay variation ranged from 2 to 4%. Urine cortisol levels were assessed by the same assay after purification over a C18 column. Samples from each patient and matched control were handled in the same run. 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 high performance liquid chromatography 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 plasma disappearance rate of cortisol, considering all plasma hormone concentrations and their dose-dependent intra-sample variance simultaneously.<sup>17</sup> 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.<sup>17</sup> 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 (*SecretionSD*) which is generally initialized as half of the data-sampling interval, and (2) a starting values for the elimination parameter, or hormone half-life.<sup>17</sup> Thus, for 10-min sampled data, the *SecretionSD* was initialized to 5-min together with a starting value for the cortisol half-life of 65-min.<sup>18</sup> To account for intrinsic errors in the estimates of hormone secretion and removal rates, the *AutoDecon* algorithm was then used to find the best fits for both parameters.<sup>18</sup> The following parameters

of the cortisol 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.* Twenty-four-hour variations in plasma cortisol concentrations were assessed by cosinor regression, an algorithm that fits a cosine function to the data using repeated nonlinear regression.<sup>19</sup> This analysis estimates an acrophase, which is the clock time during the 24 h period at which hormone concentration is maximal; a mesor, which is the average value about which the diurnal rhythm oscillates; and an amplitude, which is half the difference between the peak and nadir values of the 24 h concentration series. Furthermore, in order to assess the effects of distinct circadian time frames on possible cortisol secretion differences between patients and controls more accurately, we divided the 24 h cortisol concentration series into six equal epochs in which we compared mean cortisol concentrations between patients and controls. The time epochs were defined as follows: (I) 1630 h – 2030 h, (II) 2030 h – 0030 h, (III) 0030 h – 0430 h, (IV) 0430 h – 0830 h, (V) 0830 h – 1230 h, and (VI) 1230 h – 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.<sup>20</sup> 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.<sup>21</sup> 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  $\pm$  standard error (SE) unless otherwise specified. Unpaired  $t$  tests were used to assess group differences. Repeated measures analysis of variance (ANOVA) was used to compare mean cortisol levels between patients and controls during specific time epochs within the circadian cycle. Spearman's correlation coefficient was applied to assess all correlations. All tests were two-tailed and significance level was set at  $p < 0.05$ . Statistical analyses were performed using SPSS for Windows (release 14.0, SPSS, Inc., Chicago, IL).

## RESULTS

### *Subjects*

The HD and the control group did not differ with respect to age, gender, BMI, body fat percentage, or lean body mass (all  $p \geq 0.754$ , **Table 1**).

### *Deconvolution analysis of cortisol time series*

The average 24 h plasma cortisol concentration profiles of HD patients and controls are displayed in **Figure 1**. Illustrations of representative cortisol concentration profiles and corresponding secretion rate profiles in one HD and the matched control subject are presented in **Figure 2**. Deconvolution analysis showed that the total 24 h cortisol secretion rate was significantly higher in HD patients compared with controls ( $3490 \pm 320$  vs.  $2500 \pm 220$  nmol/L/24 h,  $p = 0.023$ ). In addition, there was also a trend for a higher total pulsatile cortisol secretion rate



( $2830 \pm 330$  vs.  $2060 \pm 180$  nmol/L/24 h,  $p = 0.058$ ). Basal cortisol secretion rate, number of secretion bursts, and mean hormone mass secreted per burst all tended to be higher as well, although these measures did not reach statistical significance ( $p \geq 0.155$ ). Details of all deconvolution-derived cortisol secretory kinetics are presented in **Table 2**.

**Table 1. Characteristics of the study population**

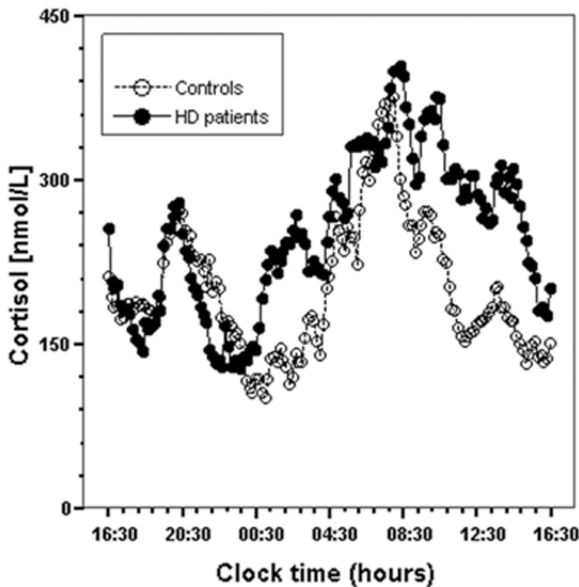
	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Male/female	5/3	5/3	-
Age (y)	46.3 (3.8)	47.6 (3.6)	0.804
BMI	23.9 (1.1)	24.2 (0.7)	0.829
Fat (%)	25.4 (2.8)	25.8 (2.7)	0.927
Lean body mass (kg)	57.1 (3.6)	55.5 (3.3)	0.754
BDI score	7.0 (1.8)	3.3 (0.8)	0.085
Mutant CAG repeat size	44.9 (1.0)	-	-
Disease duration (y)	5.1 (1.1)	-	-
UHDRS motor score	20.3 (6.4)	-	-
UHDRS behavioral score	10.5 (4.7)	-	-
TFC score	11.8 (0.8)	-	-

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) Differences between groups were assessed by unpaired t-tests.

**Abbreviations:** BDI = Beck Depression Inventory; BMI = Body Mass Index; TFC = Total Functional Capacity; UHDRS = Unified Huntington's Disease Rating Scale.

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



### Diurnal rhythmicity analysis

The results of the cosinor analysis of plasma cortisol concentration series are listed in **Table 3**. The acrophase of the cosine fit occurred in the early morning for both patients and controls and was not significantly different. However, the amplitude of the cosine function describing the diurnal plasma cortisol oscillations round the mean was significantly higher in HD patients compared with controls ( $111 \pm 14$  vs.  $64 \pm 8$  nmol/L,  $p = 0.012$ ). There was also a trend for a higher mesor in HD patients than in controls ( $p = 0.099$ ). Repeated measures ANOVA demonstrated that the mean plasma cortisol concentrations were significantly higher in HD patients compared with controls during epochs V (i.e. 0830 h – 1230 h;  $320 \pm 20$  vs.  $220 \pm 20$  nmol/L,  $p = 0.002$ ) and VI (i.e. 1230 h – 1630 h;  $250 \pm 20$  vs.  $160 \pm 20$  nmol/L,  $p = 0.007$ ), but not during epochs I to IV (i.e. 1630 h – 0830 h; all  $p \geq 0.133$ ). Therefore, using the deconvolution best fit models, we estimated pulsatile and total cortisol secretion rates for epochs I-IV and V-VI

separately. This analysis revealed that while pulsatile and total cortisol secretion rates did not significantly differ between HD and control subjects during the 1630 h to 0830 h period (pulsatile secretion:  $1860 \pm 290$  vs.

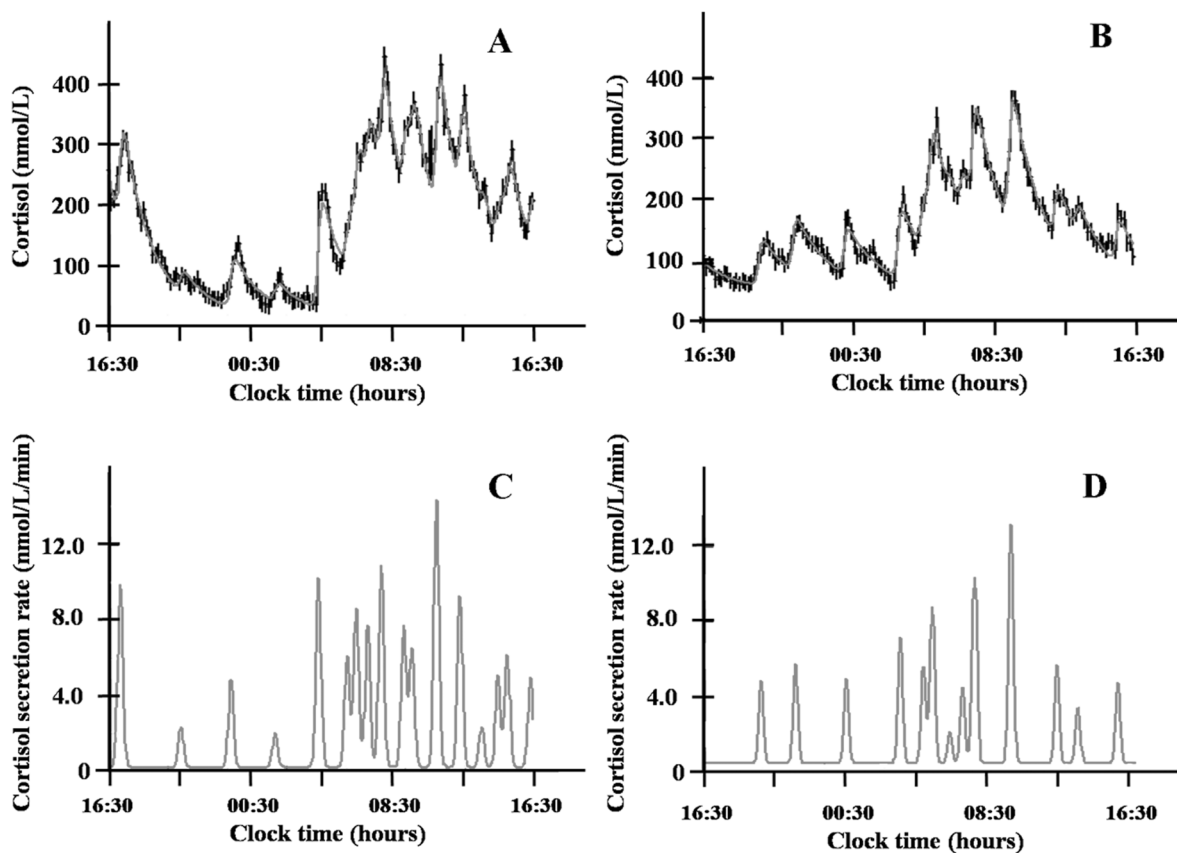
**Table 2. Deconvolution analysis of 24 h plasma cortisol concentrations.**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Half-life (min)	69.5 ± 5.1	79.6 ± 5.1	0.187
Pulse half-duration (min)	22.1 ± 4.2	15.5 ± 3.2	0.241
Pulse frequency (no./24 h)	14.4 ± 1.3	14.1 ± 0.5	0.863
Mean mass secreted per pulse (nmol/L)	250 ± 30	200 ± 20	0.155
Basal production rate (nmol/L/24 h)	0.45 ± 0.13	0.31 ± 0.06	0.340
Pulsatile production rate (nmol/L/24 h)	2830 ± 330	2060 ± 180	0.058
Total production rate (nmol/L/24 h)	3490 ± 320	2500 ± 220	0.023*
Percent pulsatile (%)	81 ± 5.1	83 ± 3.4	0.760

<sup>†</sup>) Values are indicated as mean ± SE.

<sup>‡</sup>) Differences between groups were assessed by unpaired t-tests: \* p < 0.05

**Figure 2. Deconvolution analysis of cortisol time-series.** The AutoDecon deconvolution analysis provided excellent fits to the data of individual subjects. Representative 24 h cortisol concentration profiles with fitted curves in a patient with HD (A) and the matched control subject (B). The lower panel depicts the corresponding deconvolution estimated secretory profiles in the same patient (C) and control (D).



1540 ± 200 nmol/L/24 h,  $p = 0.382$ ; total secretion: 2300 ± 290 vs. 1840 ± 210 nmol/L/24 h,  $p = 0.277$ ), they were almost two-fold higher in HD patients during the 0830 h to 1630 h period (pulsatile secretion: 970 ± 90 vs. 510 ± 60 nmol/L/24 h,  $p = 0.001$ ; total secretion: 1190 ± 80 vs. 660 ± 70 nmol/L/24 h,  $p < 0.001$ ).

### ***Regularity of plasma cortisol concentration time series***

The ApEn values of the plasma cortisol time series in HD patients were not significantly different from those in controls (0.94 ± 0.08 vs. 0.90 ± 0.06,  $p = 0.673$ ). The same held for ApEn ratios (0.51 ± 0.04 vs. 0.49 ± 0.03,  $p = 0.789$ ).

### ***Urine analysis***

**Table 3. Cosinor analysis of diurnal cortisol concentrations.**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
<b>Amplitude (nmol/L)</b>	111 ± 14	64 ± 8	0.012*
<b>Mesor (nmol/L)</b>	233 ± 24	186 ± 11	0.099
<b>Acrophase (hh:mm)</b>	07:26 ± 01:46	05:25 ± 02:19	0.503

<sup>†</sup>) Values are indicated as mean ± SE.

<sup>‡</sup>) Differences between groups were assessed by unpaired t-tests: \*  $p < 0.05$

Urine cortisol levels were also higher in HD patients, although the difference failed to reach statistical significance due to substantial inter-individual variability (114 ± 26 vs. 82 ± 13 nmol/L;  $p = 0.302$ ). There were no significant differences in urinary adrenalin, noradrenalin and dopamine levels (all  $p \geq 0.10$ ).

**Table 4. Clinical correlates of cortisol levels in Huntington's disease patients**

	Mean 24 h cortisol level [nmol/L] <sup>†</sup>
<b>Motor score</b>	0.74 (0.037) *
<b>Behavioral score</b>	-0.11 (0.779)
<b>BDI score</b>	0.15 (0.733)
<b>TFC</b>	-0.88 (0.004) **
<b>BMI</b>	-0.52 (0.037) *
<b>CAG repeat size</b>	0.20 (0.643)

<sup>†</sup>) Values are indicated as Spearman's (p-value): \*  $p < 0.05$ , \*\*  $p < 0.01$

Abbreviations: AUC = Area Under the Curve; BDI = Beck Depression Inventory; BMI = Body Mass Index; TFC = Total Functional Assessment; UHDRS = Unified Huntington's Disease Rating Scale.

### ***Cortisol levels in relation to clinical phenotype***

In HD patients, mean 24 h cortisol concentration significantly correlated with total motor score, total functional capacity and BMI (**Table 4**).

### ***Cortisol levels in relation to mutant CAG repeat size***

Although mutant CAG repeat size was not significantly related to mean cortisol levels (**Table 4**), it did significantly correlate with both 24 h pulsatile cortisol secretion rate ( $r = 0.76$ ,  $p =$

0.030) and pulse half duration ( $r = 0.85$ ,  $p = 0.007$ ). There were also trends for the associations between CAG repeat size and mean cortisol mass secreted per burst ( $r = 0.68$ ,  $p = 0.062$ ) and total 24 h cortisol secretion rate ( $r = 0.61$ ,  $p = 0.108$ ).

## DISCUSSION

We present the first detailed description of cortisol secretory dynamics in patients with HD. We found that the total 24 h cortisol production rates were significantly elevated in HD patients. The increase in cortisol production was primarily confined to the morning and early afternoon period. In addition, circadian rhythmicity analysis revealed a significantly higher amplitude of the diurnal cortisol concentration profile in HD patients. These findings point towards a disturbed central glucocorticoid feedback regulation in HD patients and indicate that HPA axis dysfunction is an early feature of the disease.

The negative feedback inhibition of cortisol production is regulated by two receptor subtypes in the brain: The high-affinity mineralocorticoid receptors (MRs) in the hippocampus that determine basal cortisol levels, and the low-affinity glucocorticoid receptors (GRs) in the hypothalamus (primarily the paraventricular nucleus (PVN)), pituitary, cortex and elsewhere in the brain, that constrain cortisol secretion during the circadian peak or acute stress.<sup>22</sup> Cortisol preferentially binds to high-affinity receptors before filling low-affinity receptors.<sup>23</sup> Therefore, the effect of MRs predominates in the early nocturnal period when cortisol levels are low, whereas the effect of GRs dominates in the morning, when cortisol levels are highest.<sup>22</sup> As in HD patients the differences in cortisol levels were mainly confined to the morning and early afternoon period, disturbed GR function is likely to underlie increased cortisol production in HD patients. Our hypothesis of diminished hypothalamic feedback as the primary cause of elevated plasma cortisol levels in HD patients would imply either decreased regularity (i.e. increased ApEn) of plasma cortisol concentration time series and/or decreased coupling between ACTH and cortisol secretion.<sup>24</sup> As cortisol ApEn did not differ between HD patients and controls, our findings are consistent with decreased coupling between ACTH and cortisol secretion in HD, however, additional experiments are required to confirm or refute this hypothesis.

Impaired GR function may result from pathology of brain structures enriched in GRs, such as the hypothalamic PVN. Indeed, there are indications for PVN pathology in HD,<sup>25,26</sup> although additional quantitative morphometric studies are needed to pinpoint its exact nature.<sup>2</sup> Transcriptional dysregulation caused by mutant huntingtin might also play a role, while direct interaction of GRs with mutant huntingtin is unlikely as suggested by Diamond et al.<sup>27</sup> In turn, loss of GR function may aggravate HD pathology as aggregation and nuclear localization of mutant huntingtin fragments containing the expanded polyglutamine stretch can be modulated by the GR, a well-characterized transcriptional regulator.<sup>27</sup> The increased diurnal amplitude of plasma cortisol levels in HD patients is also likely to be centrally mediated, resulting from pathology of hypothalamic structures directly involved in the autonomic innervation of the adrenal cortex, namely the PVN and the SCN.<sup>14,28</sup> Utilizing a neuronal pathway between the SCN, the parvocellular subdivision of the PVN (containing both autonomic and CRH neurons), and the adrenal cortex,<sup>28,29</sup> the SCN is thought to exert an inhibitory effect on cortisol release.<sup>30</sup> Indeed, apart from PVN pathology,<sup>2</sup> there are also indications that the SCN is affected in HD. Marked disruption of expression of the circadian clock genes *mPer2*, *mBmal1*, and *prokineticin 2* in the SCN have been reported in the R6/2 mouse model of HD.<sup>12,13</sup> Furthermore, the expression levels of both vasoactive intestinal

polypeptide and its receptor VPAC2 in the SCN were recently shown to be decreased in the R6/2 mice.<sup>31</sup>

Our findings extend previous reports of increased cortisol levels in HD patients<sup>7,9,10</sup> and indicate that elevated plasma cortisol levels in HD subjects are secondary to a higher pulsatile cortisol production. Moreover, we found that pulsatile cortisol secretion, which is secondary to pulsatile ACTH release and is thus thought to reflect intermittent hypothalamic drive,<sup>11</sup> correlated with mutant CAG repeat size in HD patients. This finding suggests that increased cortisol secretion in HD is likely to result from central interference of the genetic mutation with HPA axis function rather than being secondary to other neuropsychiatric features of the disease. Even mildly elevated cortisol levels are associated with a number of clinically significant health effects as illustrated by findings of, e.g., plasma lipid disturbances, hypertension, insulin resistance, and impaired cognition in patients with subclinical Cushing's syndrome and major depressive disorder in whom cortisol elevations are not as great as in the classic Cushing's syndrome.<sup>32-34</sup> Therefore, despite the absence of obvious Cushingoid features in HD patients, increased cortisol production is likely to adversely affect their health. Moreover, although our patients were all in an early stage of the disease, we found significant correlations between mean 24 h plasma cortisol levels and the UHDRS total motor score, total functional capacity and BMI, indicating that increases in cortisol levels are likely to be progressive and may become even more clinically relevant in the latter stages of the disease. Indeed, HD patients exhibit a number of symptoms and signs that might partly be attributed to hypercortisolism, such as memory deficits, mood disturbances, skeletal muscle atrophy, impaired glucose tolerance and decreased hippocampal volume.<sup>35-39</sup> However, larger scale studies are needed to confirm an association between high cortisol levels and clinical phenotype in HD patients. This is important as antiglucocorticoid therapy may also hold therapeutic potential for HD patients.<sup>40</sup>

Limitations of this study are the absence of detailed data on ACTH secretion, and dynamic tests exploring the integrity of the HPA axis. Therefore, additional experiments exploring the 24 h ACTH secretion characteristics in association with cortisol levels are required and would thus allow for the calculation of the feedback and feed-forward coupling by the cross-ApEn metrics.<sup>11</sup> In addition, challenge tests such as the dexamethasone suppression test and the CRH test are needed to more fully assess feedback and/or feed-forward effects at various levels of control within the HPA ensemble in HD patients.<sup>11</sup>

In conclusion, we found that cortisol production rate is specifically increased in the morning and early afternoon period in early-stage, medication-free HD patients. Our findings indicate disturbed central glucocorticoid feedback in HD patients, which is likely to result from pathology of the SCN and PVN nuclei of the hypothalamus.

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

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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:  $137 \pm 36$  vs.  $181 \pm 43$  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.<sup>1</sup> The disease is characterized by motor disturbances, cognitive deterioration, and psychiatric and behavioural problems.<sup>1</sup> Progressive weight loss and muscle wasting are also hallmarks of the disease, both in HD patients<sup>2-6</sup> and several transgenic mouse models of the disease.<sup>7,8</sup> 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.<sup>9,10</sup> The cause of these peripheral signs is largely unknown, although hypothalamic dysfunction, and subsequent endocrine alterations may be involved.<sup>2,11</sup>

The somatotrophic axis, which plays an essential role in body energy homeostasis, is among the hypothalamic-endocrine axes that could be affected in HD.<sup>2,11</sup> Exaggerated growth hormone (GH) responses have been observed following administration of L-dopa<sup>12</sup>, apomorphine<sup>13</sup>, bromocriptine<sup>14</sup>, arginine<sup>15,16</sup>, insulin<sup>17-19</sup> and muscimole<sup>13</sup>, whereas paradoxical GH responses have been reported after glucose<sup>12,20-22</sup>, L-dopa<sup>20</sup> or bromocriptine<sup>20,23</sup> administration. Increased mean serum GH concentrations have also been found in HD patients<sup>24,25</sup>. However, others have not been able to detect abnormalities in GH release.<sup>21,26</sup> 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.<sup>27</sup>

Elevated plasma levels of ghrelin, an orexigenic hormone of gastric origin which stimulates GH release,<sup>28</sup> have also been reported in HD patients.<sup>29,30</sup> 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.<sup>28,31</sup> 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 <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
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)	-	-

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) 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.<sup>32</sup>

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 °C for 20 min, and serum and plasma were stored at -80 °C 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 (DELFI<sup>®</sup> 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.<sup>33</sup> 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.<sup>34</sup> 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.<sup>34</sup> 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 (*SecretionSD*) which is generally initialized as half of the data-sampling interval, and (2) a starting value for the elimination parameter, or hormone half-life.<sup>34</sup> Thus, for 10-min sampled data, the *SecretionSD* was initialized to 5-min together with a starting value for the GH half-life of 16-min.<sup>35</sup> 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.<sup>35</sup> 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.<sup>36</sup> 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.<sup>36</sup> 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  $\pm$  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

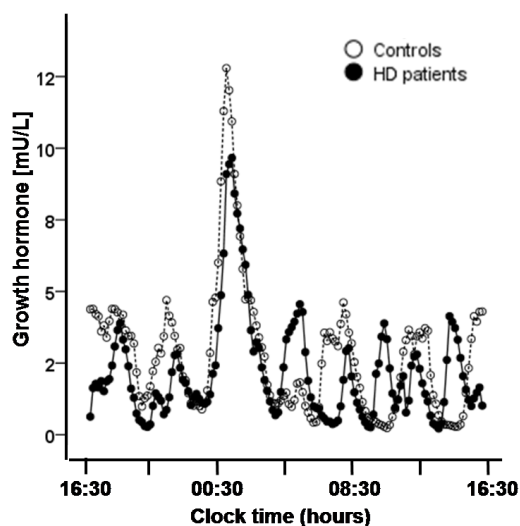
### Subjects

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 ( $2.20 \pm 0.54$  vs.  $2.83 \pm 0.59$  mU/L,  $p = 0.441$ ; **Figure 1**).

**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.



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.

### 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 ( $0.45 \pm 0.07$  vs.  $0.52 \pm 0.07$ ,  $p = 0.488$ ). The same held for ApEn ratios ( $0.39 \pm 0.03$  vs.  $0.41 \pm 0.03$ ,  $p = 0.681$ ). However, only in HD patients GH ApEn

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

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
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

<sup>†</sup>) Values are indicated as mean ± SE.

<sup>‡</sup>) 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 ( $19.01 \pm 1.25$  vs.  $20.32 \pm 2.75$  nmol/L,  $p = 0.671$ ) or IGFBP3 ( $3.97 \pm 0.23$  vs.  $3.47 \pm 0.21$  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***

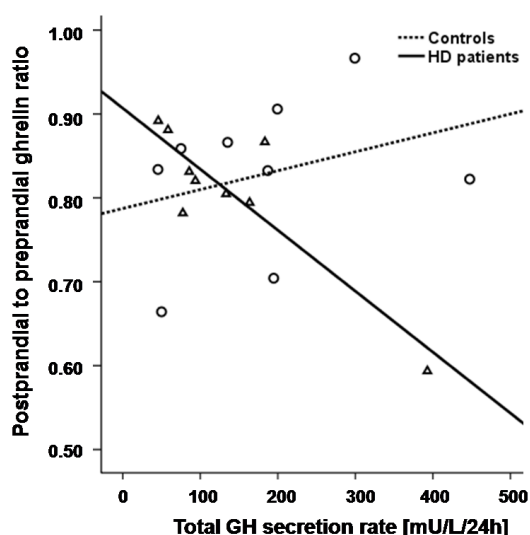
Baseline ghrelin levels (defined as the mean concentration of ghrelin in three samples obtained immediately before breakfast) were not significantly different between HD and control subjects ( $922 \pm 94$  vs.  $784 \pm 86$  pg/mL,  $p = 0.297$ ), neither were mean ghrelin levels after meal consumption ( $731 \pm 67$  vs.  $635 \pm 51$  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  $p \geq 0.177$ ) (**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  $p \geq 0.532$ ).

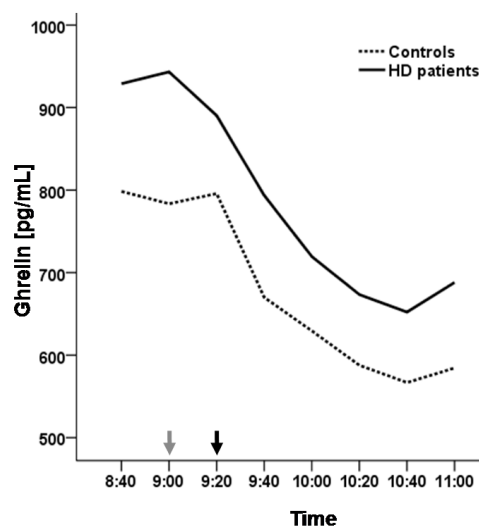
### ***GH secretion in relation to clinical phenotype***

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**),

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



**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)).



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).

### ***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 <sup>†</sup>	Controls <sup>†</sup>	HD patients <sup>†</sup>	Controls <sup>†</sup>
Body weight [kg]	-0.83**	-0.84**	0.79*	0.02
BMI [kg/m <sup>2</sup> ]	-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*	-

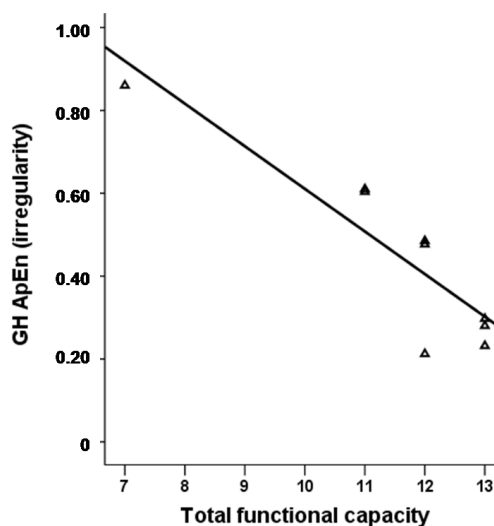
<sup>†</sup>) Values are indicated as Pearson's correlation coefficients: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**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.<sup>37</sup> 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.<sup>37</sup> 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.<sup>37</sup> Ghrelin further amplifies GH release by acting on GH-secreting cells in the pituitary, as well as GHRH and somatostatin neurons in the hypothalamus.<sup>37</sup>

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.<sup>38</sup> 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.<sup>12-23</sup>

Ghrelin secretion is thought to be triggered by the sympathetic nervous system,<sup>31</sup> whereas as yet unidentified postgastric feedback mechanisms transmitting visceral information to the brain appear to underlie meal-related suppression of plasma ghrelin.<sup>39</sup> 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 findings 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<sup>3</sup>; as ghrelin is a potent stimulator of food intake,<sup>40</sup> 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.<sup>3</sup> 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.<sup>40</sup> 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.<sup>41</sup> 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.<sup>2,11</sup> Somatostatin immunoreactivity is indeed greatly reduced in the lateral tuberal nucleus of HD patients.<sup>42</sup> However, since the somatostatin neurons in the periventricular nucleus are thought to be primarily involved in the neurohumoral regulation of GH secretion,<sup>43</sup> 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.<sup>43-45</sup> As plasma ghrelin can interact with hypocretin neurons<sup>46</sup> and hypocretin can inhibit GH secretion,<sup>47</sup> 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 somatotrophic axis strongly preserve the day to day pattern of GH release across a wide spectrum of ages and body compositions,<sup>48,49</sup> 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.

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### **Altered thyrotropic and lactotropic axes regulation in Huntington's disease**

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## ABSTRACT

**Background:** Huntington's disease (HD) is a progressive hereditary neurodegenerative disorder caused by an increased CAG repeat size in the *HTT* gene. Recently a loss of hypothalamic dopamine D<sub>2</sub> receptors was demonstrated in HD. Activation of dopamine D<sub>2</sub> receptors is known to inhibit both thyrotropic and lactotropic axes function. Hence, we postulated that loss of hypothalamic D<sub>2</sub> receptors in HD patients may give rise to disturbed thyrotropic and lactotropic axes activity, contributing to symptoms such as unintended weight loss.

**Methods:** In nine early-stage, unmedicated HD patients (6 males, 3 females) and nine age-, sex- and body mass index-matched controls, we measured serum levels of TSH and prolactin (males only) every 10 min for 24 h. Multi-parameter auto-deconvolution and approximate entropy analysis were applied to quantify basal, pulsatile and total TSH and prolactin secretion rates as well as the regularity of hormone release.

**Results:** Compared with controls, TSH and prolactin secretion tended to be slightly, but not significantly, higher in HD patients (TSH:  $1.13 \pm 0.14$  vs.  $0.91 \pm 0.19$  mU/L,  $p=0.365$ ; prolactin:  $4.91 \pm 0.42$  vs.  $4.83 \pm 0.26$   $\mu$ g/L,  $p=0.872$ ). However, in HD patients total T<sub>3</sub> and T<sub>4</sub> levels were significantly higher (T<sub>3</sub>:  $1.60 \pm 0.05$  vs.  $1.35 \pm 0.09$ ,  $p=0.027$ ; T<sub>4</sub>:  $91.9 \pm 3.9$  vs.  $81.3 \pm 3.1$ ,  $p=0.047$ ), while prolactin secretion was significantly more irregular (ApEn ratios:  $0.61 \pm 0.04$  vs.  $0.48 \pm 0.04$ ,  $p=0.037$ ). Total T<sub>3</sub> levels were negatively associated with motor impairment ( $r=-0.72$ ,  $p=0.030$ ), whereas increasing free T<sub>4</sub> levels were associated with a larger mutant CAG repeat size ( $r=+0.68$ ,  $p=0.044$ ).

**Conclusion:** Our findings indicate a mild hyperactivity of the thyrotropic axis and a disturbed regulation of the lactotropic axis in HD, both consistent with disrupted hypothalamic-pituitary dopamine signaling.

Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the *HTT* gene, resulting in a long polyglutamine tract in the N-terminus of the encoded protein huntingtin.<sup>1</sup> It is characterized by motor disturbances, cognitive decline and behavioral problems.<sup>1</sup> Progressive weight loss and muscle wasting are also hallmarks of the disease, both in HD patients<sup>2-6</sup> and several transgenic mouse models of the disease.<sup>7,8</sup> Moreover, 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.<sup>9,10</sup> The cause of these peripheral signs is largely unknown, although hypothalamic dysfunction and subsequent endocrine alterations may be involved.<sup>2,11</sup>

Both the thyrotropic and lactotropic axes are intimately involved in the complex neuroendocrine regulation of body weight and metabolism.<sup>12,13</sup> Relatively few studies have, however, evaluated hypothalamic-pituitary-thyroid axis function in HD patients. Although basal levels of total thyroxine ( $T_4$ ), triiodothyronine ( $T_3$ ), free  $T_4$ , and thyroid-stimulating hormone (TSH) have been reported to be similar between HD patients and normal controls,<sup>14,15</sup> others have found an impaired TSH response to thyrotropin-releasing hormone (TRH) stimulation.<sup>16</sup> Moreover, in a retrospective chart review study of 97 HD patients residing in long-term care facilities, the most commonly prescribed drug for problems 'unrelated' to HD was found to be levothyroxine.<sup>17</sup> Compared to thyrotropic axis function, lactotropic axis activity in HD patients has been investigated more intensively.<sup>18</sup> Nevertheless, as prolactin levels in HD patients have been reported to be unchanged,<sup>14,19-22</sup> increased,<sup>15,23</sup> or even decreased,<sup>24,25</sup> it still remains unknown whether the lactotropic axis is indeed affected in HD or whether altered prolactin levels are merely a consequence of anti-dopaminergic medication use in HD. The discordances in findings regarding thyrotropic and lactotropic axes functioning in HD are likely due to the use of a few baseline measurements of hormone levels or long blood sampling intervals which are not adequate to assess either the pulsatile nature of TSH and prolactin secretion or their total daily production rates.<sup>26</sup>

Recently a loss of hypothalamic dopamine  $D_2$  receptors was demonstrated in both early stage HD patients as well as premanifest HD mutation carriers.<sup>27</sup> Activation of dopamine  $D_2$  receptors is known to inhibit both thyrotropic and lactotropic axes function.<sup>28,29</sup> Therefore, we postulated that loss of hypothalamic  $D_2$  receptors in HD patients may give rise to disturbed thyrotropic and lactotropic axes activity, contributing to the disrupted energy homeostasis in these subjects. We tested this hypothesis by deconvolution analysis of 24 h serum TSH and prolactin concentration profiles as well as assessment of thyroid hormone levels in both early stage, medication-free 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. Thyroid axis function was assessed in all participants. However, as estrogens can have a marked impact on prolactin secretion,<sup>12</sup> lactotropic axis activity was assessed in male subjects only. 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 symptoms and signs.<sup>30</sup> None of the subjects used medication, except one male HD patient who discontinued paroxetine ( $t_{1/2} \approx 21$  h)

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 medical 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. Blood was allowed to clot and, within 60 min of sampling, all tubes were centrifuged at 4000 rotations/min at 4 °C for 20 min, and plasma was stored at -80 °C 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, catecholamines and cortisol 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*

**Table 1. Characteristics of the study population**

	HD patients <sup>1</sup>	Controls <sup>1</sup>	p-value <sup>2</sup>
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)	-	-

<sup>1</sup>) Values are indicated as mean (SE).

<sup>2</sup>) Differences between groups were assessed by unpaired t-tests.

**Abbreviations:** BMI = Body Mass Index; TFC = Total Functional Capacity; UHDRS = Unified Huntington's Disease Rating Scale.

Serum TSH and prolactin levels were measured by time-resolved immunofluorometric assays (Delfia, Wallac Oy, Turku, Finland). The detection limit of the TSH assay was 0.01 mU/L, and the interassay variation ranged from 3.1 to 8.3% at very low levels. The detection limit of the prolactin assay was 0.05 µg/L, and the interassay variation ranged from 2.7 to 3.8%. Serum T<sub>4</sub> and T<sub>3</sub> levels were measured with Abbott AxSYM (Abbott Laboratories, Abbott Park, IL). Free T<sub>4</sub> concentrations were estimated using electrochemiluminescence immunoassays (Roche Diagnostic Nederland BV, Almere, The Netherlands). Urinary epinephrine, norepinephrine and dopamine concentrations were assessed by high performance liquid chromatography 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 disappearance rate of TSH and prolactin, considering all plasma hormone concentrations and their dose-dependent intra-sample variance simultaneously.<sup>31-33</sup> The standard deviation of the secretion events was initialized to 5-min. For TSH, a fixed two-component half-life was assumed with 18-min for the first component and 92-min for the second component, with a relative contribution of 32% of the slow component to the total elimination.<sup>34</sup> For prolactin, a starting one-component half-life of 45-min was assumed,<sup>35</sup> and the *AutoDecon* algorithm was then used to find the best fit.<sup>36</sup> The following parameters of the TSH and prolactin 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.* Twenty-four-hour variations in plasma hormone concentrations were assessed by cosinor regression, an algorithm that fits a cosine function to the data using repeated nonlinear regression.<sup>37</sup> This analysis estimates an acrophase, which is the clock time during the 24 h period at which hormone concentration is maximal; a mesor, which is the average value about which the diurnal rhythm oscillates; and an amplitude, which is half the difference between the peak and nadir values of the 24 h concentration series.

*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.<sup>38</sup> 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.<sup>39</sup> 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  $\pm$  standard error (SE) unless otherwise specified. Unpaired  $t$  tests were used to assess differences in means between the two groups. Pearson's correlation coefficient was applied to assess all correlations. 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 urinary creatinine, epinephrine, norepinephrine and dopamine levels (all  $p \geq 0.10$ ).

#### *Deconvolution analysis of TSH time series*

Mean 24 h TSH and prolactin concentrations were not significantly different between HD patients and controls (TSH:  $1.13 \pm 0.14$  vs.  $0.91 \pm 0.19$  mU/L,  $p = 0.365$ ; prolactin:  $4.91 \pm 0.42$  vs.  $4.83 \pm 0.26$  µg/L,  $p = 0.872$ ; **Figure 1**). The number of TSH and prolactin pulses as well as their basal, pulsatile and total secretion rates were also similar in the patient and control group (all  $p \geq 0.342$ ). Details of all deconvolution-derived SH and prolactin secretory kinetics are presented in **Table 2**.

#### *Diurnal rhythmicity analysis*

TSH and prolactin displayed significant diurnal variations, both in patients and in controls (**Figure 1**). However,

**Table 2. Deconvolution analysis of 24 h serum TSH prolactin concentrations.**

	TSH		Prolactin	
	HD patients <sup>1</sup>	Controls <sup>1</sup>	HD patients <sup>1</sup>	Controls <sup>1</sup>
<b>Basal secretion rate<sup>2</sup></b>	16.8 (1.8)	12.8 (2.6)	75.4 (27.8)	44.8 (13.1)
<b>Pulsatile secretion rate<sup>2</sup></b>	10.4 (1.9)	9.2 (2.5)	59 (11)	62 (7)
<b>Total secretion rate<sup>2</sup></b>	27.2 (3.6)	22.0 (4.7)	134 (38)	106 (17)
<b>Percent pulsatile [%]</b>	36.0 (3.0)	37.8 (4.4)	48.5 (4.5)	61.5 (7.5)
<b>Pulse half-duration [min]</b>	50.3 (5.7)	57.2 (9.7)	41.5 (11.4)	30.3 (9.0)
<b>Pulse frequency [no./24 h]</b>	11.4 (1.2)	10.9 (1.4)	15.7 (1.2)	17.3 (1.2)
<b>Mean mass secreted per pulse<sup>3</sup></b>	0.94 (0.20)	0.87 (0.22)	4.2 (1.2)	3.8 (0.7)

<sup>1</sup>) Values are indicated as mean (SE). There were no significant differences between HD patients and controls (unpaired t-tests).

<sup>2</sup>) Secretion rates are in mU/L/24 h for TSH, and ug/L/24 h for prolactin.

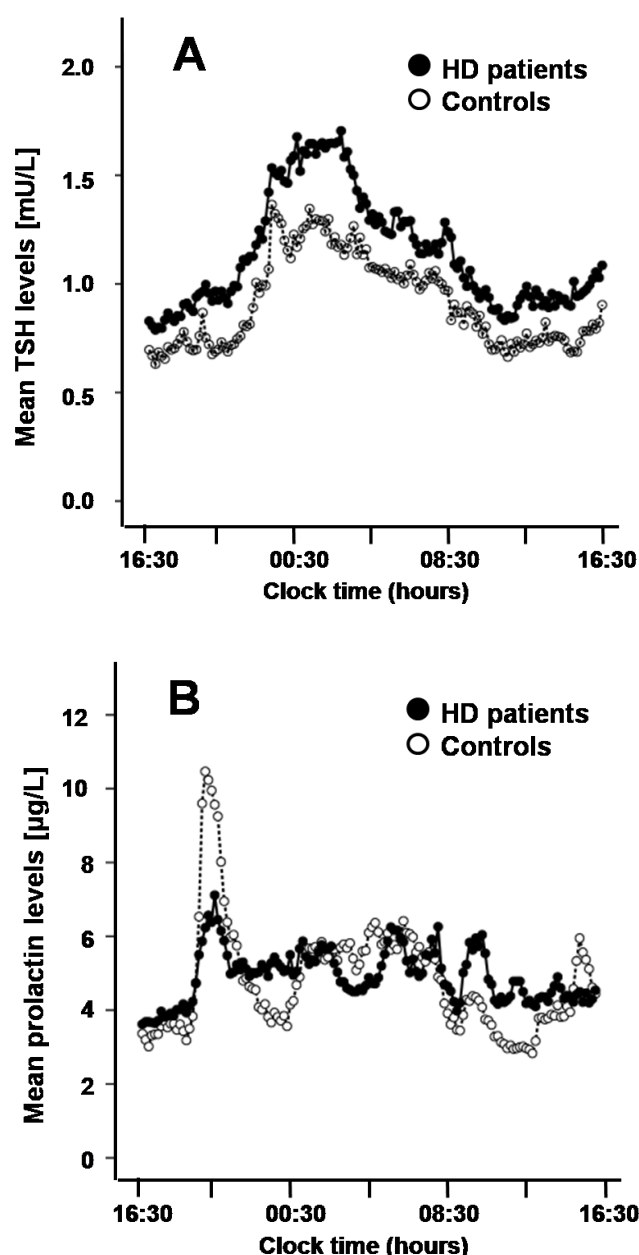
<sup>3</sup>) Mean mass secreted per pulse is in mU/L for TSH, and ug/L for prolactin.

the acrophase, amplitude and mesor of the TSH and prolactin concentration series were not significantly different between HD patients and controls (all  $p \geq 0.353$ ).

#### *Regularity of TSH and prolactin concentration time series*

The ApEn values and ApEn ratios of the TSH time series were similar between HD patients and controls (both  $p \geq 0.712$ ). However, the ApEn values of the prolactin time series were significantly higher in HD patients compared with controls ( $1.06 \pm 0.08$  vs.  $0.80 \pm 0.09$ ,  $p = 0.048$ ). The same held for ApEn ratios ( $0.61 \pm 0.04$  vs.  $0.48 \pm 0.04$ ,  $p = 0.037$ ), indicating significantly more irregular prolactin secretion in HD patients (**Figure 2**).

**Figure 1. Mean serum TSH (A) and prolactin (B) concentrations in HD and control subjects. Sampling started at 1630 h and was continued at 10-min intervals for 24 h.**



were similar between HD patients and matched controls, thyroid hormone levels were significantly higher in HD patients, consistent with a mild hyperactivity of the hypothalamic-pituitary-thyroid axis. Total daily prolactin production rates were also similar between HD patients and controls, however, prolactin secretion was significantly more irregular in HD patients.

Thyroid hormones ( $T_4$  and  $T_3$ ) are critically involved in the regulation of systemic energy homeostasis and

### Thyroid hormone levels

Fasting levels of both  $T_3$  and  $T_4$  were significantly higher in HD patients compared with controls ( $T_3$ :  $1.60 \pm 0.05$  vs.  $1.35 \pm 0.09$ ,  $p = 0.027$ ;  $T_4$ :  $91.9 \pm 3.9$  vs.  $81.3 \pm 3.1$ ,  $p = 0.047$ ). However, while free  $T_4$  levels also tended to be higher in HD patients ( $15.1 \pm 0.70$  vs.  $14.2 \pm 0.46$ ), the difference did not reach statistical significance ( $p = 0.343$ ).

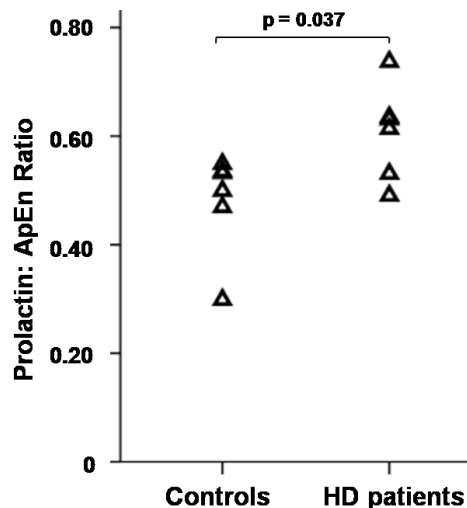
### Thyrotropic and lactotropic axes activity in relation to clinical phenotype

In HD patients, total daily TSH and prolactin secretion rates were not significantly associated with BMI, motor score, total functional capacity or mutant CAG repeat size (all  $p \geq 0.17$ ). However, higher total  $T_3$  levels were significantly associated with less motor impairment ( $r = -0.72$ ,  $p = 0.030$ ). Moreover, increasing mutant CAG repeat size was significantly related to higher free  $T_4$  levels ( $r = +0.68$ ,  $p = 0.044$ ). Trends also existed for the associations between total  $T_4$  levels and mutant CAG repeat size ( $r = +0.63$ ,  $p = 0.069$ ), and total  $T_4$  levels and BMI ( $r = -0.64$ ,  $p = 0.064$ ).

## DISCUSSION

In this study we rigorously evaluated thyrotropic and lactotropic axes function in a group of early stage, medication-free HD patients. Although daily TSH production rates

**Figure 2. Prolactin secretion regularity in HD patients and controls.** Compared with controls, the approximate entropy (ApEn) ratio of prolactin secretion is higher in HD patients ( $0.48 \pm 0.04$  vs.  $0.61 \pm 0.04$ ,  $p=0.037$ ), indicating significantly more irregular prolactin release.



their secretion is tightly regulated by a complex interplay of positive and negative feedback loops.<sup>40</sup> Hypothalamic TRH induces pituitary TSH secretion which then stimulates the synthesis and release of thyroid hormones by the thyroid gland. Although TSH synthesis and secretion are primarily controlled by the stimulatory action of TRH and the negative feedback restraint by thyroid hormones, other factors such as dopamine exert important modulatory effects.<sup>40</sup> Dopamine has a dual influence on TSH secretion: it inhibits TSH synthesis and release through  $D_2$  receptor activation at the level of the pituitary thyrotropes, whereas it stimulates TRH secretion by the hypophysiotropic neurons located in the paraventricular nucleus.<sup>29</sup> Using positron emission tomography with  $^{11}\text{C}$ -raclopride, a specific  $D_2$  receptor ligand, Politis et al.<sup>27</sup> recently demonstrated a loss of  $D_2$  receptors in the hypothalamus of both early stage HD patients and premanifest HD mutation carriers. Moreover, in the R6/2 transgenic mouse model of HD a loss of  $D_2$  receptors has also been

reported at the level of the anterior pituitary.<sup>41</sup> Therefore, our finding of a mild thyrotropic axis hyperactivity in HD patients may, at least partly, be attributed to a specific pattern of hypothalamic and pituitary  $D_2$  receptor loss. The modest decrease of hypothalamic  $D_2$  receptors (by about 28%) in early stage HD patients,<sup>27</sup> may explain the relatively mild increase in the activity of the thyrotropic axis in our cohort.

Altered hypothalamic-pituitary dopamine signaling may also underlie the significantly more irregular pattern of prolactin release in HD patients. There is now ample evidence that dopamine of tubero-infundibular origin, delivered through long portal vessels into the sinusoid capillaries of the anterior pituitary, is the major physiological regulator of prolactin release.<sup>12</sup> Hypothalamic dopamine inhibits the basally high-secretory tone of pituitary lactotrophs by binding to  $D_2$  receptors expressed on their cell membranes.<sup>28</sup> Prolactin in turn regulates the activity of the tubero-infundibular neurons via a short-loop feedback mechanism.<sup>28</sup> Hence, pathology of the tubero-infundibular dopaminergic system, located in the hypothalamic infundibular nucleus (i.e. the human homologue of the arcuate nucleus in rodents), or loss of pituitary  $D_2$  receptor expression as described in the R6/2 mice<sup>41</sup> could both underlie the irregular pattern of prolactin secretion in HD patients. The diminished regularity of prolactin secretion even in our cohort of early, unmedicated HD patients may also account for the inconsistencies in findings from previous studies on prolactin levels in HD subjects,<sup>14,15,19-25</sup> since due to the irregular pattern of prolactin release single or a few baseline measurements are likely to yield ambiguous outcomes. It remains to be established to what extent the irregular pattern of prolactin secretion in HD could lead to abnormal responses to physiological stimuli of prolactin release such as stress.<sup>12</sup> Neuropathological

evaluation of the infundibular nucleus, as well as *in vivo* assessment of pituitary D<sub>2</sub> receptor binding in HD patients could provide more mechanistic insights into the basis of this abnormality in prolactin secretion.

Interestingly, when assessed in relation to clinical characteristics, higher free T<sub>4</sub> levels were associated with larger mutant CAG repeat sizes. In addition, there was an inverse trend for the relation between total T<sub>4</sub> levels and BMI in HD patients. As thyroid hormones are known to increase energy expenditure, elevated thyroid hormone levels in early stage HD patients that seem to increase with mutant CAG repeat size, may contribute to the lower BMI in HD mutation carriers,<sup>5,6</sup> and possibly account for the association between mutant *HTT* CAG repeat size and weight loss in HD.<sup>3</sup> As mutant CAG repeat size was not associated with TSH secretion, its association with free T<sub>4</sub> levels is more likely to be mediated peripherally, for example, by a direct effect of mutant huntingtin on tissue deiodinases that are found throughout the body.<sup>13,42</sup> However, larger scale studies in, especially early stage and neuroleptic-free, HD patients are needed to confirm these preliminary associations.

In conclusion, we found a mild hyperactivity of the hypothalamic-pituitary-thyroid axis, as well as a more irregular pattern of prolactin secretion in HD patients compared with matched controls. These findings are consistent with disrupted hypothalamic-pituitary dopamine signaling in HD. Further neuropathological, imaging and functional studies are necessary to unveil the cause of these abnormalities and provide rationale for potential endocrine-based therapies for HD.

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### **Leptin secretion rate increases with higher CAG repeat number in Huntington's disease patients**

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## ABSTRACT

*Background.* Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an increased number of CAG repeats in the *huntingtin* gene. A hallmark of HD is unintended weight loss, the cause of which is unknown. *Objective.* To perform a detailed analysis of adipose tissue function in HD patients as abnormal fat tissue function could contribute to the weight loss. *Design, setting & participants.* In a clinical research laboratory, twenty-four-hour plasma concentrations of leptin, adiponectin and resistin were studied in nine early-stage, medication-free HD patients and nine age-, sex- and body mass index (BMI)-matched controls. *Measurements.* Leptin was measured every 20 min whereas adiponectin and resistin were measured hourly. Auto-deconvolution and cosinor regression were applied to quantify secretion characteristics of leptin and diurnal variations in leptin, adiponectin and resistin levels. *Results.* Plasma levels and diurnal rhythmicity of leptin, adiponectin and resistin were not significantly different between HD patients and controls. However, although leptin production increased with higher BMI and fat mass in controls, no such relation was present in HD patients. Moreover, when corrected for fat mass, mean plasma leptin concentration as well as basal, pulsatile and total secretion rates increased with the size of the CAG repeat mutation ( $r=+0.72$  to  $r=+0.80$ ; all  $p<0.05$ ). Both higher pulsatile leptin secretion and higher mean adiponectin levels were associated with a greater degree of motor and functional impairment in HD patients. *Conclusions.* CAG repeat size dependent interference of the HD mutation with adipose tissue function may contribute to weight loss in HD patients.

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder caused by an increased number of CAG repeats in the *huntingtin* gene.<sup>1</sup> It is characterized by motor disturbances, cognitive decline and behavioral problems.<sup>1</sup> Unintended weight loss is also a hallmark of the disease, both in HD patients<sup>2-5</sup> and several mouse models of the disease.<sup>6,7</sup> Weight loss frequently leads to general weakening and a decline in the quality of life of HD patients.<sup>8</sup> On the other hand, a higher Body Mass Index (BMI) has been associated with a slower rate of disease progression.<sup>9</sup> The cause of weight loss in HD is unknown, although decreased caloric intake, increased motor activity or a higher metabolic rate, due to both central and peripheral defects, may be involved.<sup>2,3</sup>

Interestingly, weight loss in HD patients is accompanied by substantial loss of body fat stores.<sup>4,10,11</sup> Moreover, abnormal fat cell function has been reported in several mouse models of HD.<sup>6,12,13</sup> As adipose tissue and release of endocrine and paracrine factors, so called 'adipokines', by fat cells take center stage in the regulation of feeding and body weight<sup>14</sup>, defects in fat metabolism may partly contribute to weight loss in HD patients.<sup>6</sup> However, adipokine secretion characteristics have hardly been studied in patients with HD. Single baseline measurements of leptin, the most important adipokine, have yielded conflicting results, showing either unchanged or decreased levels in HD patients.<sup>15-19</sup> Moreover, there are no reports available on leptin secretory dynamics and diurnal variation, both of which are thought to be essential for normal hormone function<sup>20</sup>; particularly in light of recent reports of substantial circadian rhythm disturbances in HD<sup>21</sup>, these aspects should be accounted for. In addition, levels of the two other major adipokines, i.e. adiponectin and resistin, have not been assessed so far in HD patients.

We hypothesized that altered adipose tissue function may contribute to weight loss in HD patients. Thus, in order to assess adipose tissue function and its relation to body weight in HD patients, we assessed 24 h plasma leptin, adiponectin and resistin concentration profiles in both early-stage HD patients and matched healthy control subjects. Moreover, we assessed whether mutant *huntingtin* could interfere with adipokine secretion in a CAG repeat size dependent manner, which might account for our recent finding of increased weight loss in HD patients with a higher mutant CAG repeat size.<sup>3</sup>

## 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 symptoms and signs.<sup>22</sup> All subjects were free of 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

**Table 1. Characteristics of the study population**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
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)	-	-

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) 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.

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 20-min intervals. Blood was allowed to clot and, within 60-min of sampling, all tubes were centrifuged at 4000 rotations/min at 4 °C for 20 min, and plasma was stored at -80 °C 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). Twenty-four hour urine was collected for the determination of creatinine and catecholamine concentrations. Subjects remained sedentary except for bathroom visits. 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 (BIA) was used to assess fat mass, lean body mass and fat percentage at 0800 h.

### *Assays*

Plasma leptin, adiponectin and resistin were all measured by radioimmunoassay (Linco Research, St. Charles, MO, USA). The coefficients of variation ranged from 3.0 to 5.1% for leptin, 6.3 to 8.1% for adiponectin, and 3.2 to 5.4% for resistin. The detection limits of the assays were 0.5 µg/L for leptin, 1.0 mg/L for adiponectin, and 0.15 µg/L for resistin. Samples from each patient and matched control were handled in the same run. 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 high performance liquid chromatography with electron capture detection (ESTA-Coulochem, Chelmsford, MA, USA).

## Calculations and statistics

*Deconvolution analysis.* A recently developed, fully automatic, multiparameter deconvolution procedure, *AutoDecon*, was used to estimate various specific measures of secretion and plasma disappearance rate of leptin, considering all plasma hormone concentrations and their dose-dependent intra-sample variance simultaneously.<sup>23</sup> 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.<sup>23</sup> 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 (*SecretionSD*) which is generally initialized as half of the data-sampling interval, and (2) and an estimate of the elimination parameter, or hormone half-life.<sup>23</sup> Thus, for 20-min sampled data, the *SecretionSD* was initialized to 10-min, while a fixed two-component leptin half-life was assumed with 3.4-min for the first component and 71-min for the second component, with a relative contribution of 81% of the slow component to the total elimination (J.D. Veldhuis, personal communication). The following parameters of the leptin time series were estimated: number of secretory bursts, secretory burst half-duration (duration at half-maximal amplitude), mean mass secreted per burst, basal secretion rate, pulsatile secretion rate, and total secretion rate. As adiponectin and resistin levels were assessed hourly, deconvolution of the adiponectin and resistin time series was not possible.

*Diurnal rhythmicity analysis.* Twenty-four hour variations in plasma leptin, adiponectin and resistin concentrations were assessed by cosinor regression, an algorithm that fits a cosine function to the data using repeated nonlinear regression. This analysis estimates an acrophase, which is the clock time during the 24 h period at which hormone concentration is maximal; a mesor, which is the average value about which the diurnal rhythm oscillates; and an amplitude, which is half the difference between the peak and nadir values of the 24 h concentration series.

*Statistical analysis.* Results are expressed as mean  $\pm$  standard error (SE) unless otherwise specified. Unpaired *t* tests were used to assess group differences. Pearson's correlation coefficient was applied to assess all correlations. All tests were two-tailed and significance level was set at  $p < 0.05$ . Statistical analyses were performed using SPSS for Windows (release 14.0, SPSS, Inc., Chicago, IL).

## RESULTS

### Subjects

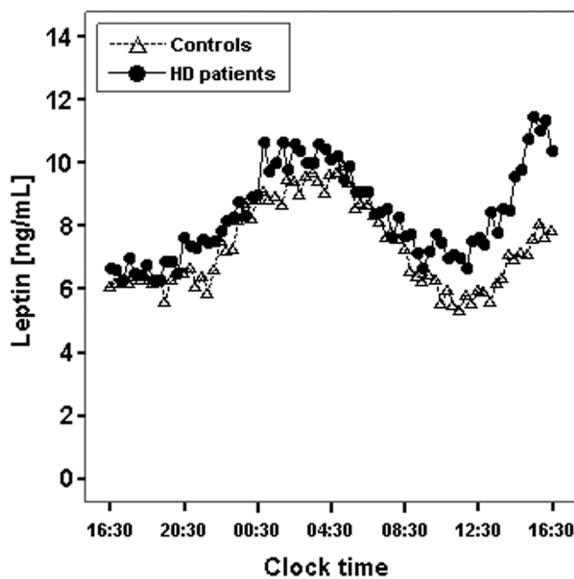
The HD and the control group did not differ with respect to age, sex, BMI, body fat percentage, or lean body mass (all  $p \geq 0.15$ , Table 1). There were also no significant differences in urinary creatinine, epinephrine, norepinephrine and dopamine levels (all  $p \geq 0.10$ ).

### Leptin levels and deconvolution analysis of leptin time series

The average 24 h plasma leptin concentration profiles of HD patients and controls are displayed in Figure 1. Mean 24 h leptin concentration did not differ significantly between patients and controls ( $8.4 \pm 3.1$  vs.  $7.4 \pm 3.2$

$\mu\text{g/L}$ ,  $p = 0.823$ ). Basal, pulsatile and total leptin secretion rates were also not significantly different between the two groups (all  $p \geq 0.785$ ), although they tended to be higher in HD patients. Details of all deconvolution-derived leptin secretory kinetics are presented in Table 2. Results were similar when leptin levels and secretory dynamics in each individual were expressed per kilogram body fat (data not shown).

**Figure 1.** Mean plasma leptin concentrations in HD patients and matched control subjects. Sampling started at 1630 h and was continued at 20-min intervals for 24 h.



#### Adiponectin and resistin levels

The average 24 h plasma adiponectin and resistin concentration profiles of HD patients and controls are shown in Figure 2. Although throughout the circadian cycle adiponectin levels were higher and resistin levels were lower in HD patients compared with controls, the two groups did not differ significantly with respect to mean 24 h levels of adiponectin ( $9.6 \pm 1.9$  vs.  $8.0 \pm 1.6$  mg/L,  $p = 0.540$ ) or resistin ( $12.0 \pm 1.7$  vs.  $13.8 \pm 1.6$   $\mu\text{g/L}$ ,  $p = 0.469$ ). Results remained similar when expressed per kilogram body fat (data not shown).

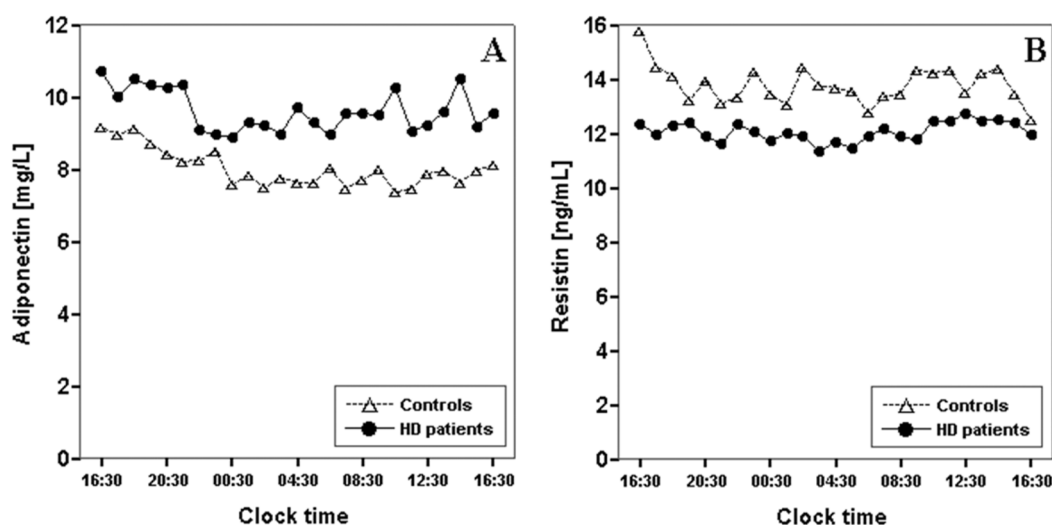
**Table 2.** Deconvolution analysis of 24 h plasma leptin concentrations.

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Basal secretion rate [ $\mu\text{g/L/24 h}$ ]	0.08 (0.03)	0.07 (0.03)	0.791
Pulsatile secretion rate [ $\mu\text{g/L/24 h}$ ]	35 (12)	30 (13)	0.785
Total secretion rate [ $\mu\text{g/L/24 h}$ ]	147 (55)	125 (54)	0.786
Percent pulsatile [%]	24.2 (2.0)	22.3 (2.8)	0.576
Pulse half-duration [min]	39.8 (8.4)	51.3 (5.8)	0.276
Pulse frequency [no./24 h]	6.4 (0.4)	5.6 (0.8)	0.889
Mean mass secreted per pulse [ $\mu\text{g/L}$ ]	5.0 (1.6)	5.0 (2.1)	0.992

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) Differences between groups were assessed by unpaired t-tests.

**Figure 2.** Mean plasma adiponectin (A) and resistin (B) concentrations in HD patients and matched control subjects. Assessment of plasma adiponectin and resistin levels started at 1630 h and was continued at 60-min intervals for 24 h.



### Diurnal rhythmicity analysis

The results of the cosinor analysis of plasma leptin, adiponectin and resistin concentration series are listed in Supplementary Table 1. Leptin, adiponectin and resistin levels displayed significant diurnal variations, both in patients and controls. The acrophases of leptin, adiponectin and resistin concentration series occurred at similar time points in the patient and the control group and were not significantly different. Similarly, the amplitude and mesor of all three concentration series were not significantly different between the two groups (Supplementary Table 1).

### Leptin, adiponectin and resistin levels in relation to clinical phenotype

Comparison of the correlations between leptin production, and BMI and body fat stores in HD patients and control subjects revealed a number of differences between the two groups. Unlike in controls, mean 24 h leptin levels in HD patients did not significantly increase with higher BMI ( $r = +0.67$  and  $p = 0.048$  in controls vs.  $r = +0.43$  and  $p = 0.25$  in patients). Moreover, in controls basal, pulsatile and total leptin secretion rates per kilogram fat increased with higher fat mass, whereas in HD patients these associations were much weaker and not significant (Table 3). When adjusted for fat mass, mean 24 h resistin levels also significantly decreased with higher BMI in controls but not in HD patients (Table 3).

In the HD group, mean 24 h leptin as well as basal, pulsatile and total leptin secretion rates per kilogram fat all significantly increased with the size of the CAG repeat expansion in the mutant huntingtin gene (Table 3). Also mean 24 h adiponectin per kilogram fat increased with higher CAG repeat size, however, this relation failed to reach statistical significance. In addition, higher mean 24 h adiponectin as well as higher pulsatile leptin secretion rate per kilogram fat were significantly associated with a greater degree of motor and functional impairment as measured by scores on the UHDRS total motor, total functional capacity, functional assessment



and independence scales (Table 3).

**Table 3. Clinical correlates of adipokine levels in Huntington's disease patients and controls**

	Leptin								Mean adiponectin levels <sup>†</sup>		Mean resistin levels <sup>†</sup>	
	Mean levels <sup>†</sup>		Basal secretion rate <sup>†</sup>		Pulsatile secretion rate <sup>†</sup>		Total secretion rate <sup>†</sup>		HD	C	HD	C
	HD	C	HD	C	HD	C	HD	C				
<b>BMI</b>	-0.06	0.58	0.00	0.64	-0.31	0.38	-0.09	0.57	-0.47	-0.40	-0.48	-0.80*
<b>Fat mass [kg]</b>	0.37	0.82**	0.43	0.87**	0.08	0.68*	0.36	0.82**	-0.19	0.00	-0.59	-0.64
<b>Body fat [%]</b>	0.72*	0.90**	0.76*	0.92**	0.48	0.82**	0.69*	0.90**	-0.21	0.25	-0.64	-0.37
<b>CAG repeat size</b>	0.75*	-	0.72*	-	0.80**	-	0.77*	-	0.51	-	0.08	-
<b>Motor score</b>	0.55	-	0.49	-	0.75*	-	0.57	-	0.73*	-	-0.13	-
<b>TFC</b>	-0.46	-	0.38	-	-0.72*	-	-0.49	-	-0.79*	-	0.18	-
<b>FAS</b>	-0.46	-	-0.51	-	-0.80**	-	-0.60	-	-0.80**	-	0.23	-
<b>IS</b>	-0.50	-	-0.43	-	-0.72*	-	-0.52	-	-0.75*	-	0.31	-

<sup>†</sup>) For each individual all parameters were divided by kilograms of body fat to correct for fat mass. Values are indicated as Pearson's correlation coefficients: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Abbreviations:** BMI = Body Mass Index; C = Control subjects; FAS = Functional Assessment; HD = Huntington disease patients; IS = Independence Score; TFC = Total Functional Capacity.

## DISCUSSION

Here we present the first detailed description of leptin secretory dynamics and its diurnal variation in HD patients. In addition, we provide the first description of adiponectin and resistin levels in these patients. We found that there are no significant differences in the levels or diurnal rhythmicity of these adipokines between HD patients and controls. However, when corrected for fat mass, both mean plasma leptin concentration and secretion rate significantly increased with the size of the CAG repeat mutation in HD patients. Moreover, higher pulsatile leptin secretion and mean adiponectin levels were associated with a greater degree of clinical impairment. Interestingly, unlike in controls, neither BMI nor body fat mass was significantly related to leptin production in HD patients. These findings suggest that the HD mutation interferes with adipose tissue function and are important for understanding the cause of weight loss in HD.

The adipose-tissue derived hormone leptin is produced in proportion to body fat stores and its circulating levels serve to communicate body energy states to the central nervous system where it inhibits food intake and stimulates energy expenditure.<sup>14</sup> Numerous studies have shown that circulating leptin levels as well as leptin gene expression per gram lipid weight increase with higher BMI and fat mass.<sup>24,25</sup> However, here we demonstrate that in HD patients such a relation is not apparent. This is in line with findings from another study which did not find a correlation between the plasma leptin concentration in a single fasting blood sample and BMI in HD patients, while the correlation was highly significant in control subjects.<sup>17</sup> Moreover, we found a significant association between leptin production rate and mutant CAG repeat size. As expression of an inducible mutant *huntingtin* transgene in an adipocyte cell line impaired gene expression and lipid accumulation<sup>6</sup>, it is likely that mutant huntingtin directly interferes with leptin production in a polyglutamine length-dependent manner, thereby confounding the relation between body fat content and leptin levels in HD patients.

Recently, we demonstrated that the rate of weight loss in HD patients increases with a higher number of

CAG repeats in the mutant *huntingtin* gene.<sup>3</sup> Similarly, R6/2 mice with larger mutant CAG repeat lengths in the transgene had a lower body weight.<sup>3</sup> Here we show that the production rate of leptin, an anorexigenic hormone that stimulates energy expenditure<sup>14</sup>, also increases with higher CAG repeat number. Therefore, enhanced leptin production rate in HD patients with higher CAG repeat lengths in the mutant allele could lead to decreased appetite and hypermetabolism, thereby contributing to the higher rate of weight loss in these subjects. In humans, leptin is encoded by the LEP gene, the promoter of which contains consensus sequence binding sites for the transcriptional activator specificity protein-1 (Sp1).<sup>26</sup> In HD, gene microarray studies have indicated selective transcriptional alterations of many genes that contain binding sites for Sp1<sup>27,28</sup>, which has been shown to interact with huntingtin in a polyglutamine length-dependent manner.<sup>29</sup> Up-regulation of Sp1 is thought to occur in response to mutant huntingtin, whereas down-regulation of Sp1 has been associated with neuroprotection in both *in vitro* and *in vivo* HD models.<sup>30</sup> Therefore, the underlying mechanisms through which a higher CAG repeat number is related to increasing leptin levels in HD patients may involve polyglutamine length-dependent transcriptional dysregulation. Indeed, interrogation of a publicly available microarray database revealed that LEP gene expression is higher in HD brains compared with controls<sup>27</sup>, although the difference did not reach statistical significance likely because compared with adipose tissue LEP gene expression is much lower in the brain.

Leptin is the only adipocyte the concentration of which has been reported previously in HD patients. However, while some investigators found decreased leptin levels<sup>16,17</sup>, others reported no change.<sup>15,18,19</sup> Differences in age, gender, BMI and circadian timing of the measurements between patients and controls have likely been responsible for these contradictory results. Here we accounted for these differences by measuring plasma leptin levels throughout 24 h in a homogenous group of early-stage, medication-free HD patients and a group of age-, gender- and BMI-matched controls. We demonstrate that although mean 24 h leptin levels and production rate in early-stage HD patients do not differ from those in controls, within the HD group there is substantial variability in leptin levels which is largely due to differences in mutant CAG repeat size. Although age and gender are related to leptin levels, this association is assumed to be almost exclusively due to age- and gender-specific changes in body fat content.<sup>24</sup> Therefore, as we corrected leptin secretion for fat mass, age and gender heterogeneity within the HD group are unlikely to have influenced the association between leptin secretion and mutant CAG repeat size (Table 3).

We also found that the levels of adiponectin and resistin, two other adipose tissue-specific hormones that are implicated in energy homeostasis and glucose and lipid metabolism<sup>14</sup>, are not significantly different between HD patients and controls. However, adiponectin levels corrected for fat mass did increase with disease severity in HD patients. Therefore, differences in adiponectin levels may become more marked in the later stages of the disease and, thus, might serve as a biomarker to track disease progression in HD.

In conclusion, our findings suggest subtle abnormalities in adipose tissue function in early-stage HD patients that are possibly due to polyglutamine length-dependent interference of mutant huntingtin with transcriptional mechanisms. These abnormalities may aggravate with disease progression and could contribute to weight loss in HD patients.

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**Supplementary table 1. Cosinor analysis of diurnal leptin, adiponectin and resistin concentrations.**

		<b>HD patients<sup>†</sup></b>	<b>Controls<sup>†</sup></b>	<b>p-value<sup>‡</sup></b>
<b>Leptin</b>	<b>Amplitude [µg/L]</b>	1.2 (0.4)	1.6 (0.7)	0.617
	<b>Mesor [µg/L]</b>	8.4 (3.1)	7.4 (3.2)	0.825
	<b>Acrophase [hh:mm]</b>	02:22 (01:04)	03:35 (00:40)	0.355
<b>Adiponectin</b>	<b>Amplitude [mg/L]</b>	0.6 (0.1)	0.6 (0.2)	0.982
	<b>Mesor [mg/L]</b>	9.6 (1.9)	8.0 (1.6)	0.535
	<b>Acrophase [hh:mm]</b>	05:07 (01:06)	06:13 (00:48)	0.428
<b>Resistin</b>	<b>Amplitude [µg/L]</b>	0.6 (0.1)	1.1 (0.2)	0.087
	<b>Mesor [µg/L]</b>	12.0 (1.7)	13.8 (1.6)	0.472
	<b>Acrophase [hh:mm]</b>	04:56 (01:06)	03:27 (01:19)	0.401

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) Differences between groups were assessed by unpaired t-tests.

## **Delayed onset of the diurnal melatonin rise in patients with Huntington's disease**

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## ABSTRACT

Sleep disturbances are very prevalent in Huntington's disease (HD) patients and can substantially impair their quality of life. Accumulating evidence suggests considerable dysfunction of the hypothalamic suprachiasmatic nucleus (SCN), the biological clock, in both HD patients and transgenic mouse models of the disease. As melatonin has a major role in the regulation of sleep and other cyclical bodily activities and its synthesis is directly regulated by the SCN, we postulated that disturbed SCN function is likely to give rise to abnormal melatonin secretion in HD. Therefore, we compared 24h melatonin secretion profiles between early-stage HD patients and age-, sex- and body mass index-matched controls. Although mean diurnal melatonin levels were not different between the two groups ( $p=0.691$ ), the timing of the evening rise in melatonin levels was significantly delayed by more than one and a half hours in HD patients ( $p=0.048$ ). Moreover, diurnal melatonin levels strongly correlated with both motor ( $r=0.70$ ,  $p=0.036$ ) and functional impairment ( $r=0.78$ ,  $p=0.013$ ). These findings suggest a delayed sleep phase syndrome-like circadian rhythm disorder in early stage HD patients and suggest that melatonin levels may progressively decline with advancing disease.



Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat size in the gene encoding the protein huntingtin.<sup>4</sup> The disease is characterized by motor impairment, cognitive deterioration, behavioral problems and progressive weight loss.<sup>4</sup> With an estimated prevalence of nearly 90% disturbed sleep is also a prominent feature of the disease, substantially impairing the quality of life of both patients and caregivers.<sup>20</sup> Sleep disturbances in HD patients include an increased sleep onset latency, frequent nocturnal awakenings, reduced sleep efficiency, delayed and shortened rapid eye movement sleep, and increased periodic leg movements.<sup>1,8,22,24</sup> Moreover, wrist actigraphy studies indicate circadian rhythm disturbances in HD patients, which are mirrored in the most widely used transgenic mouse model of the disease, the R6/2 mouse.<sup>10,14,15</sup> The R6/2 mice show progressive disruption of the day-night activity patterns, with increased daytime activity and a concurrent decrease in nocturnal activity, eventually leading to a complete disintegration of the circadian behavior.<sup>14,15</sup> Interestingly, disrupted circadian behavior in these mice is accompanied by marked dysregulation of expression of a number of circadian clock genes in the hypothalamic suprachiasmatic nucleus (SCN), the principal rhythm generating system in mammals.<sup>12,14,15</sup> Furthermore, we recently demonstrated an increased amplitude of the diurnal cortisol profile as well as an increased rate of early day cortisol production in HD patients, both of which are also consistent with SCN dysfunction in HD.<sup>3</sup>

Melatonin is a hormone that is primarily secreted at night by the pineal gland and has a major role in the regulation of sleep and other cyclical bodily activities.<sup>5,16</sup> Melatonin synthesis is directly regulated by the SCN via a multisynaptic pathway in response to the environmental light/dark cycle, and thus, melatonin is considered an endogenous humoral synchronizer that signals 'time of day' to all tissues throughout the body.<sup>5</sup> Conversely, the two major melatonin receptors, MT1 and MT2 receptors, are abundantly expressed in the SCN and are thought to mediate melatonin's sleep-promoting and circadian phase-shifting effects.<sup>5</sup> Therefore, we postulated that disturbed SCN function in HD is likely to give rise to abnormal melatonin secretion, which in turn could contribute to impaired sleep and circadian rhythm disturbances in HD patients. In addition, as apart from its timekeeping functions melatonin has also strong antioxidative properties, abnormal melatonin secretion may also influence the neurodegenerative process underlying HD. In order to test these hypotheses, we (1) compared 24 h plasma melatonin concentration profiles between early stage, medication-free HD patients and healthy matched controls, and (2) assessed the association between mean diurnal melatonin levels and clinical phenotype.

## 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**. 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 symptoms and signs.<sup>9</sup>

All subjects were free of medication, except one HD patient who discontinued paroxetine use

three weeks prior to study. 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 every hour. Blood was allowed to clot and, within 60-min of sampling, centrifuged at 4000 rotations/min at 4 °C for 20 min, and plasma was stored at -80 °C 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). Twenty-four hour urine was collected for the determination of creatinine and catecholamine concentrations. Subjects remained sedentary except for bathroom visits. No daytime naps were allowed. Lights were switched off at 2300 h and back on at 0730 the next morning.

### *Assays*

Plasma melatonin was measured by radioimmunoassay (Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, GER). The detection limit of the assay was 2 pg/mL. The intra-assay and interassay variations ranged from 9.8 to 12.3% and from 9.6 to 16.2%, respectively. Samples from each patient and matched control were handled in the same run. 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 high performance liquid chromatography with electron capture detection (ESTA-Coulochem, Chelmsford, MA, USA).

### *Analysis of melatonin profiles*

Individual diurnal variations of plasma melatonin levels were quantified by a best-fit curve obtained

**Table 1. Characteristics of the study population**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Male/female	6/3	6/3	-
Age [y]	47.1 (3.4)	48.6 (3.3)	0.691
BMI	24.1 (1.0)	24.3 (0.6)	0.691
Mutant CAG repeat size	44.4 (1.0)	-	-
Disease duration [y]	5.7 (1.1)	-	-
UHRS motor score	22.2 (6.0)	-	-
TFC score	11.7 (0.7)	-	-
Functional Assessment	23.3 (0.7)	-	-
Independence score	94.4 (2.8)	-	-

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) Differences between groups were assessed by the Mann-Whitney *U*-test.

**Abbreviations:** BMI = Body Mass Index; FAS = Functional Assessment; TFC = Total Functional Capacity; UHRS = Unified Huntington's Disease Rating Scale.

using locally weighted linear regression with a Gaussian kernel and a regression window of four hours as previously described.<sup>11</sup> For each melatonin profile the nadir and the acrophase were defined as the minimum and maximum of the best-fitting curve, while the amplitude was defined as half of the difference between the acrophase and nadir values. The onset of the melatonin rise was defined as the timing of the first plasma level exceeding the mean + 3 standard deviations (SDs) of baseline levels recorded over the 1030-1430 period, not followed to lower concentrations before the acrophase. The melatonin offset was defined as the timing of the last value occurring after the acrophase that exceeded +3 SDs of the baseline values.<sup>11</sup>

### Statistical analysis

Results are expressed as mean  $\pm$  standard error (SE) unless otherwise specified. The non-parametric Mann-Whitney *U*-test was used to assess group differences. Spearman's correlation coefficient was applied to assess all correlations. 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

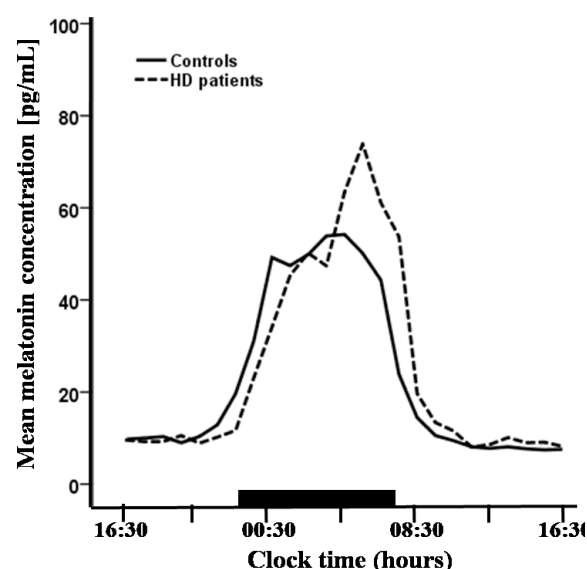
### Subjects

The HD and the control group did not differ with respect to age, sex, and BMI (all  $p \geq 0.691$ , **Table 1**). There were also no significant differences in urinary creatinine, epinephrine, norepinephrine and dopamine levels (all  $p \geq 0.10$ ).

### Melatonin profiles

Mean 24 h melatonin levels were not significantly different between HD patients and controls ( $24.8 \pm 5.4$  vs.  $22.7 \pm 2.8$  pg/mL,  $p = 0.691$ ; **Figure 1**). Also the acrophase and nadir concentrations, as well as the amplitude of the diurnal melatonin profile were not significantly different between the two groups ( $p \geq 0.857$ ; **Table 2**). In one HD patient, however, the diurnal melatonin profile was extremely irregular and at no point did melatonin concentrations rise above three standard deviations of the mean baseline values (this subject also happened to be the most severely affected patient with scores of 63 and 7 on the UHDRS motor and total functional capacity subscales, respectively). Consequently, melatonin onset, offset and duration could not be defined in this subject; therefore, for subsequent comparison of these parameter values between the two groups also the data of the matched control subject were excluded from the analyses.

**Figure 1. Mean 24 h melatonin levels in HD patients and matched controls.** The diurnal melatonin rise was significantly delayed in HD patients by about one and a half hours ( $p=0.048$ ). The black bar on the abscissa indicates the dark period (23:00-7:30 h).



**Table 2. Melatonin secretion characteristics in HD patients and controls.**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Mean 24 h levels (pg/mL)	24.8 (5.4)	22.7 (2.8)	0.601
Acrophase conc. (pg/mL)	76.6 (20.4)	60.1 (9.7)	0.860
Nadir conc. (pg/mL)	5.8 (0.9)	5.8 (0.7)	0.857
Amplitude (pg/mL)	35.4 (9.9)	27.2 (4.8)	0.895
Onset time (hh:mm)	00:30 (00:22)	22:52 (00:40)	0.048*
Offset time (hh:mm)	07:30 (00:48)	08:22 (00:21)	0.478
Nocturnal duration (h)	7.0 (1.0)	9.5 (0.8)	0.063

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) Differences between groups were assessed by the Mann-Whitney *U*-test; \* *p* < 0.05.

Melatonin onset time was significantly delayed in HD patients compared with controls (00:30 h  $\pm$  22 min vs. 22:52 h  $\pm$  37 min, *p* = 0.048). Melatonin offset time, however, was similar between the two groups (Table 2). Consequently, there was also a trend for a shorter duration of the

nocturnal plasma melatonin plateau in HD patients (6.2  $\pm$  1.2 vs. 9.1  $\pm$  0.8 h, *p* = 0.063).

### *Melatonin levels and clinical phenotype*

In HD patients, mean 24 h melatonin levels significantly correlated with UHDRS motor score (*r* = -0.70, *p* = 0.036), total functional capacity (*r* = + 0.78, *p* = 0.013), and independence score (*r* = + 0.88, *p* = 0.002), but not with mutant CAG repeat size (*r* = +0.18, *p* = 0.645).

## DISCUSSION

Here we present the first detailed description of diurnal melatonin profiles in HD patients. We show that the timing of the evening rise in melatonin levels is significantly delayed by more than one and a half hours in these patients compared with matched control subjects. Moreover, despite similar mean diurnal melatonin levels between our early stage HD patients and controls, we found strong inverse associations between mean diurnal melatonin levels and both motor and functional disability in these patients, suggesting that decreases in melatonin levels are likely to become more pronounced in the later stages of the disease.

Delayed onset of melatonin secretion in HD patients is reminiscent of a delayed sleep phase syndrome (DSPS)-like circadian rhythm disorder.<sup>25</sup> The pathophysiological basis of DSPS is assumed to lie in a slower endogenous clock with an abnormally long intrinsic circadian periodicity, resulting in a delayed phase position of the overt circadian rhythms, including those of melatonin, cortisol and core body temperature.<sup>25</sup> Interestingly, recently we also found an increased rate of early day cortisol production in HD patients, which may also be a manifestation of delayed circadian rhythms in HD.<sup>3</sup> Circadian rhythm disturbances in HD are likely to stem directly from pathology within the SCN molecular oscillation, caused either by the toxic effects of mutant huntingtin locally and/or arising from dysfunction of brain circuitry afferent to the SCN.<sup>14,15</sup> In favour of local pathology is the detection of neuronal inclusions of mutant huntingtin in the SCN of HD patients<sup>2</sup>, as well as the finding of SCN dysfunction at both mRNA and protein level in R6/2 mice, with reduced levels of the positive regulator *mBmal1* and truncated peak expression of its target genes *mPer1*, *mPer2*, and *mProk2*.<sup>14</sup> However, intact oscillation of SCN neurons from R6/2 mice *in vitro*, when released from the pathological context, is consistent

with an afferent cause of SCN dysfunction in HD.<sup>15</sup> Alternatively, impaired expression and/or function of the melatonin receptors in HD could play a role, particularly the MT2 receptor subtype which is enriched in the SCN and is known to be involved in phase-shifting of the biological clock.<sup>17</sup> Regardless of the cause of diurnal rhythm disturbances in HD, however, restoration of circadian rhythms by pharmacological imposition of sleep has been shown to improve cognitive decline in R6/2 mice, suggesting that a similar strategy may be beneficial to HD patients. As our findings indicate a DSPS-like phenotype in early stage HD, another approach that may be evaluated in these patients is to treat them with melatonin and/or bright light at the appropriate times so as to phase advance the clock.<sup>13,25</sup> The administration of melatonin at the subjective dusk, and the use of bright light at the subjective dawn and avoidance of light in the subjective evening, could be used to phase advance the clock.<sup>13,25</sup>

Interestingly, we also found that mean diurnal melatonin levels in HD patients decreased with increasing severity of the clinical phenotype, suggesting that melatonin levels may decline substantially with advancing disease course. Progressive abnormalities in the metabolism of the melatonin precursor tryptophan may account for this association,<sup>7,19</sup> although additional investigations are needed to pinpoint the exact underlying metabolic pathways. Numerous studies have shown the ability of melatonin and its kynuramine metabolites to increase the survival of neurons under conditions of enhanced oxidative stress.<sup>18</sup> Therefore, declining melatonin levels may contribute to the progressive neurodegeneration in HD, and conversely, exogenous melatonin supplementation may be of benefit to HD patients. In fact, melatonin can antagonise the cytotoxic properties of both quinolinic acid and 3-nitropropionic acid, the administration of which is used to model HD induced pathology.<sup>6,21</sup> Moreover, recently it was demonstrated that melatonin can potently inhibit mitochondrial cytochrome *c* release, which is known to activate downstream cell death pathways, resulting in neuroprotection in a mutant huntingtin expressing striatal cell line.<sup>23</sup>

In conclusion, our findings suggest a DSPS-like circadian rhythm disorder in early stage HD patients and indicate that melatonin levels may progressively decline with advancing disease. Therefore, strategies aimed at advancing the phase of the biological clock as well as melatonin supplementation might be of benefit to HD patients. However, first larger scale studies are needed to confirm our findings and to assess whether later stages of HD are also accompanied by a similar circadian rhythm disorder.

## ACKNOWLEDGMENTS

We are greatly indebted to: Prof Dr J.H. Meijer for her valuable suggestions and comments, and E. J. M. Ladan-Eygenraam, and E. C. Sierat-van der Steen for technical assistance during the study. N.A.Aziz is supported by The Netherlands Organisation for Scientific Research (grant #017.003.098).

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## PART IV

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# **M**ETABOLIC STUDIES IN **H**UNTINGTON'S DISEASE





*“Diabetes. Ligate pancreatic ducts of dog. Keep dogs alive till acini degenerate leaving Islets. Try to isolate the internal secretion of these to releave glycosuria.”*

**Frederick Grant Banting** *Banting’s notebook, October 31, 1920, 2:00 AM.* In: Bliss M. *The Discovery of Insulin.* Chicago: University of Chicago Press; 1982, p. 50.



## **Systemic energy homeostasis in Huntington's disease patients**

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## ABSTRACT

**Background.** Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an increased number of CAG repeats in the *HTT* gene. Apart from neurological impairment, the disease is also accompanied by progressive weight loss, abnormalities in fat and glucose homeostasis and a higher prevalence of diabetes mellitus, the causes of which are unknown. Therefore, we aimed to perform a detailed analysis of systemic energy homeostasis in HD patients in relation to disease characteristics. **Methods.** Indirect calorimetry combined with a hyperinsulinemic-euglycemic clamp with stable isotopes ([6,6- $^2\text{H}_2$ ]-glucose and [ $^2\text{H}_5$ ]- glycerol) was performed to assess energy expenditure, and glucose and fat metabolism in nine early-stage, medication-free HD patients and nine age-, sex- and body mass index-matched controls. **Results.** Compared with controls, fasting energy expenditure was higher in HD patients ( $1616 \pm 72$  vs.  $1883 \pm 93$  kCal/24 h,  $p=0.037$ ) and increased even further after insulin stimulation ( $1667 \pm 87$  vs.  $2068 \pm 122$  kCal/24 h,  $p=0.016$ ). During both basal and hyperinsulinemic conditions, glucose and glycerol disposal rates, endogenous glucose production and hepatic insulin sensitivity were similar between HD patients and controls. In HD patients, energy expenditure increased with disease duration, but not with a greater degree of motor or functional impairment. Moreover, a higher mutant CAG repeat size was associated with lower insulin sensitivity ( $r=-0.84$ ,  $p=0.018$ ). **Conclusion.** These findings suggest sympathetic hyperactivity as an underlying mechanism of increased energy expenditure in HD, as well as peripheral polyglutamine-length dependent interference of mutant huntingtin with insulin signaling that may become clinically relevant in carriers of mutations with large CAG repeat sizes.

Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the *HTT* gene, resulting in a long polyglutamine tract in the N-terminus of the encoded protein huntingtin.<sup>1</sup> The disease is characterized by motor disturbances, cognitive deterioration, and psychiatric and behavioural problems.<sup>2</sup> Progressive weight loss and muscle wasting, despite sustained or even increased caloric intake, are also hallmarks of the disease, both in HD patients<sup>3-5</sup> and several transgenic mouse models of the disease.<sup>6-9</sup> Moreover, 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.<sup>7,10,11</sup> These peripheral abnormalities may not only considerably impair the quality of life of HD patients but could also affect the neurodegenerative process.<sup>12,13</sup> However, the cause of the peripheral signs in HD is largely unknown, although both hypothalamic dysfunction and peripheral defects in glucose and fat metabolism may be involved.<sup>3,13,14</sup>

Several studies have implicated transcriptional repression of the peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) by mutant huntingtin in HD associated neurodegeneration.<sup>15-17</sup> The transcriptional coactivator PGC-1 $\alpha$  is a potent regulator of mitochondrial biogenesis and respiration, and therefore, its downregulation by mutant huntingtin is thought to provide the long-sought link between transcriptional dysregulation and mitochondrial dysfunction in HD.<sup>18</sup> Interestingly, recently it was shown that mutant huntingtin also inhibits transcriptional activity of PGC-1 $\alpha$  and its target genes in peripheral tissues such as muscle and fat in both HD patients and animal models of the disease.<sup>19,20</sup> As PGC-1 $\alpha$  has emerged as a master regulator of systemic energy homeostasis involved in adaptive thermogenesis,  $\beta$ -oxidation of fatty acids, insulin sensitivity and carbohydrate metabolism in general,<sup>21</sup> we hypothesized that impaired peripheral PGC-1 $\alpha$  activity in HD patients is likely to give rise to disturbances of fat and glucose metabolism and diminish insulin sensitivity.

Systemic substrate metabolism and its responsiveness to insulin stimulation has, however, not yet been rigorously assessed in HD patients. Hence, in order to elucidate the underlying mechanisms of weight loss and impaired glucose homeostasis in HD, in this study we aimed to accurately assess resting energy expenditure, as well as lipid and glucose metabolism during both basal and insulin stimulated conditions in early stage, medication-free HD patients in comparison with matched controls.

## SUBJECTS AND METHODS

### *Subjects*

Nine early-stage HD patients (stages I and II) 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 symptoms and signs.<sup>22</sup> None of the subjects used medication, except one HD patient who discontinued paroxetine (20 mg/day;  $t_{1/2} \approx 21$  h) 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 condition 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 Medical Ethics Committee of the Leiden University Medical Center.

**Table 1. Characteristics of the study population**

	HD patients <sup>†</sup>	Controls <sup>†</sup>	p-value <sup>‡</sup>
Male/female	6/3	6/3	-
Age [y]	47.5 (3.4)	48.8 (3.3)	0.797
BMI	23.6 (0.8)	24.0 (0.6)	0.700
Fat [%]	24.5 (2.4)	24.6 (2.1)	0.972
Lean body mass [kg]	57.5 (3.6)	56.4 (3.0)	0.818
Waist-to-hip ratio	0.92 (0.02)	0.94 (0.02)	0.591
Mutant CAG repeat size	44.4 (1.0)	-	-
Disease duration [y]	6.1 (1.1)	-	-
UHDRS motor score	22.2 (6.0)	-	-
TFC score	11.7 (0.7)	-	-

<sup>†</sup>) Values are indicated as mean (SE).

<sup>‡</sup>) 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.

### **Clinical protocol**

All studies started at 8:00 AM after an overnight fast. Height, weight, BMI and waist-to-hip ratio were measured according to the World Health Organization recommendations. Lean body mass and fat percentage were assessed by bioelectrical impedance analysis. Metabolic studies were performed as described previously.<sup>23</sup> In short, patients were requested to lie down on a bed in a semirecumbent position. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was inserted into a contralateral dorsal hand vein for blood sampling; this hand was kept in a heated box (60 °C) throughout the test to obtain arterialized blood. Samples were taken for the measurement of basal levels of glucose, insulin, glucagon, cholesterol, triglycerides, nonesterified fatty acids (NEFAs), glycerol, and background enrichment of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose and [<sup>2</sup>H<sub>5</sub>]- glycerol. At 8:30 AM (t = 0 min), an adjusted primed (17.6 μmol/kg × actual plasma glucose concentration [mmol/L]) continuous (0.33 μmol/kg per minute) infusion of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose (enrichment 99.9%; Cambridge Isotopes, Cambridge, Mass) was started and continued throughout the study. After 60 minutes, a primed (1.6 μmol/kg) continuous (0.11 μmol/kg) infusion of [<sup>2</sup>H<sub>5</sub>]-glycerol (Cambridge Isotopes) was started and continued throughout the study. During this period, indirect calorimetry with a ventilated hood (Oxycon Beta, Mijnhardt Jaegher, Breda, The Netherlands) was performed for 30-min for basal glucose and lipid oxidation rates.<sup>24</sup> At the end of the basal period, four blood samples were taken at 10-min intervals for the determination of plasma glucose, glycerol, insulin, and [6,6-<sup>2</sup>H<sub>2</sub>]-glucose – and [<sup>2</sup>H<sub>5</sub>]-glycerol –specific activities. Subsequently, a primed continuous infusion of insulin (Actrapid, Novo Nordisk Pharma BV, Alphen aan de Rijn, The Netherlands; 40 mU/m<sup>2</sup>/min) was started (t = 120 min). Exogenous glucose 20% enriched with 3% [6,6-<sup>2</sup>H<sub>2</sub>]-glucose was infused at a variable rate to maintain the plasma glucose level at 5.0 mmol/L. A second indirect calorimetry was performed at the end of the hyperinsulinemic clamp. From t = 210 to 240 min, blood was drawn every 10 min for the determination of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose – and [<sup>2</sup>H<sub>5</sub>]-glycerol –specific activities, glucose, insulin, and glycerol. While blood in the serum samples was allowed to clot, plasma samples were immediately put on ice. Within 60 min of sampling, all samples were centrifuged at



1610 g at 4 °C for 20 min, and then stored at -80 °C until assay.

### *Assays*

Serum insulin and glucagon were measured with an immunoradiometric assay (Biosource, Nivelles, Belgium) and a radioimmunoassay (Linco Research, St. Charles, MO, USA), respectively. Serum triacylglycerol was measured with a fully automated Modular P800 Hitachi system (Tokyo, Japan). Serum NEFAs were assessed by an enzymatic colorimetric acyl-CoA synthase/oxidase assay (Wako Chemicals, Neuss, Germany), while serum glucose, [6,6-<sup>2</sup>H<sub>2</sub>]-glucose, and [<sup>2</sup>H<sub>5</sub>]-glycerol were determined in a single analytical run using gas chromatography-mass spectrometry as described previously.<sup>23</sup>

### *Calculations*

A physiological and isotopic steady-state was achieved during the last 30 min of both the basal as well as the hyperinsulinemic period; therefore, the rates of appearance and disappearance for glucose and glycerol were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio.<sup>25</sup> Glucose flux rates were expressed per kg fat free mass, whereas glycerol flux rates were normalized per kg fat mass. Endogenous glucose production (EGP) during the basal steady-state is equal to the rate of appearance of glucose, whereas EGP during the clamp was calculated as the difference between the rates of glucose appearance and infusion. Since the fasting plasma insulin concentration is a strong inhibitory stimulus for EGP, the basal hepatic insulin resistance index ( $\mu\text{mol}/\text{min}/\text{kg}_{\text{FFM}}/\text{pmol} \times \text{L}$ ) was calculated as the product of fasting EGP and fasting plasma insulin concentration.<sup>26</sup> The metabolic clearance rate of insulin was calculated as the constant infusion rate of insulin divided by the steady-state serum insulin concentration corrected for endogenous insulin secretion. Total lipid and carbohydrate oxidation rates were calculated as described by.<sup>24</sup> Non-oxidative glucose metabolism was calculated by subtracting the glucose oxidation rate (determined by indirect calorimetry) from glucose rate of disappearance.

### *Statistical analysis*

Results are expressed as mean  $\pm$  standard error (SE) unless otherwise specified. Unpaired *t* tests were used to assess differences in means between the two groups, whereas paired *t* tests were applied to assess mean differences between basal and hyperinsulinemic conditions. Partial rank correlation coefficients were used to assess all correlations while adjusting for the effects of age and sex. 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) and TANAGRA (release 1.4, Lyon, France).

## **RESULTS**

### *Subjects*

The HD and the control group did not differ with respect to age, sex, BMI, body fat percentage, or lean body mass (all  $p \geq 0.70$ , Table 1). Moreover, fasting levels of glucose, insulin, glycosylated hemoglobin (HbA<sub>1c</sub>), glucagon, triglycerides, cholesterol and NEFAs were similar between the two groups (all  $p \geq 0.10$ , Table 2).

**Table 2. Metabolic parameters in patients with Huntington's disease and matched controls during basal and hyperinsulinemic conditions**

	Basal Conditions			Hyperinsulinemia		
	HD patients	Controls	<i>p</i> value	HD patients	Controls	<i>p</i> value
Energy Expenditure (kCal/24 h)	1883 ± 93	1616 ± 72	0.037*	2068 ± 122	1667 ± 87	0.016*
Glucose $R_d$ (μmol/kg <sub>FFM</sub> /min)	20.7 ± 0.8	21.2 ± 0.6	0.381	65.0 ± 7.2	68.6 ± 9.2	0.884
Glucose Oxidation (μmol/min)	653 ± 137	709 ± 102	0.748	1783 ± 227	1314 ± 68	0.065
NOGD (μmol/kg <sub>FFM</sub> /min)	12.7 ± 4.3	7.6 ± 1.7	0.290	33.6 ± 6.9	40.0 ± 7.8	0.543
EGP (μmol/kg <sub>FFM</sub> /min)	20.7 ± 0.8	21.2 ± 0.6	0.381	13.7 ± 1.0	15.0 ± 1.6	0.850
HIR (μmol kg <sub>FFM</sub> /min/ pmol × L)	1176 ± 126	1232 ± 83	0.547	7841 ± 549	7891 ± 595	0.558
Glycerol $R_a$ (μmol/kg <sub>FM</sub> /min)	7.4 ± 0.9	6.4 ± 0.8	0.409	2.6 ± 0.4	2.3 ± 0.3	0.503
Lipid Oxidation (μmol/min)	340 ± 31	252 ± 26	0.045*	93 ± 47	107 ± 23	0.785
Plasma insulin (mU/l)	9.2 ± 0.9	8.8 ± 1.1	0.758	82.4 ± 3.1	76.0 ± 2.9	0.156
MCR <sub>I</sub> (ml/m <sup>2</sup> /min)	-	-	-	0.56 ± 0.02	0.60 ± 0.2	0.181
Glucose (mmol/l)	4.6 ± 0.2	4.6 ± 0.2	0.875	4.9 ± 0.1	4.8 ± 0.2	0.566
Glycerol (mmol/l)	50.0 ± 6.9	50.9 ± 8.4	0.938	21.8 ± 7.2	17.8 ± 2.9	0.618
HbA1c (%)	4.8 ± 0.1	4.9 ± 0.1	0.579	-	-	-
Glucagon (ng/l)	39.8 ± 3.3	51.9 ± 6.1	0.103	-	-	-
NEFA (mmol/l)	0.51 ± 0.07	0.40 ± 0.04	0.200	-	-	-
Triacylglycerol (mmol/l)	1.0 ± 0.2	0.9 ± 0.1	0.662	-	-	-
Total cholesterol (mmol/l)	4.4 ± 0.2	4.0 ± 0.6	0.525	-	-	-

Data are means ± SEM. \*  $p < 0.05$

**Abbreviations:** EGP = endogenous glucose production; FFM = fat free mass; FM = fat mass; HIR = hepatic insulin resistance; MCR<sub>I</sub> = metabolic clearance rate of insulin; NEFA = non-esterified fatty acids; NOGD, non-oxidative glucose disposal;  $R_a$  = rate of appearance;  $R_d$  = rate of disappearance

### Energy expenditure

Basal resting energy expenditure was significantly higher in HD patients compared with controls (1883 ± 93 vs. 1616 ± 72 kCal/24 h,  $p = 0.037$ ), which was mainly due to a higher rate of lipid oxidation (Table 2). Insulin stimulation caused a significant rise in the resting energy expenditure in HD patients, but not in controls (Figure 1, Table 2). Although insulin stimulation induced a significant rise in glucose oxidation rate in both groups, the magnitude of this rise was greater in HD patients (1130 ± 194 vs. 605 ± 110 μmol/min,  $p = 0.032$ ).

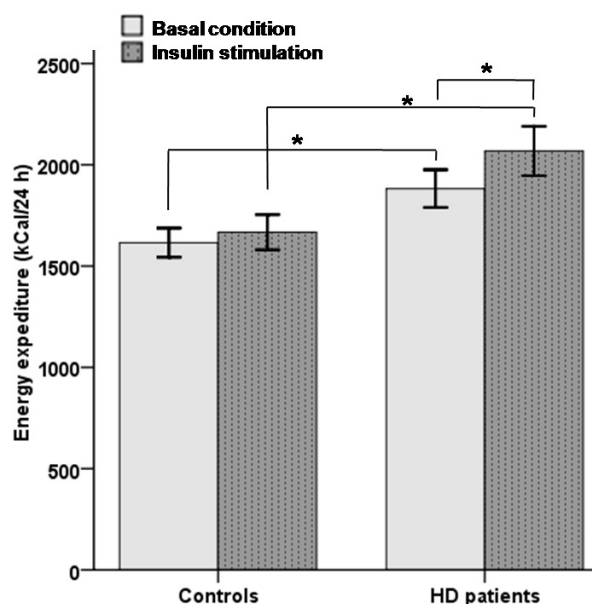
### Glucose metabolism

Both during basal and hyperinsulinemic conditions whole body glucose disposal rate, as well as non-oxidative glucose disposal, endogenous glucose production and hepatic insulin resistance were similar between HD patients and controls (Table 2). Insulin stimulation significantly increased glucose disposal and suppressed glucose production, however, there were no significant differences between HD patients and controls (Table 2).

### Lipid metabolism

Plasma glycerol levels as well as the rate of appearance of glycerol, which is a measure of lipolysis, were similar between HD patients and controls. Moreover, hyperinsulinemia suppressed lipolysis to a similar degree in both groups (Table 2).

**Figure 1. Energy expenditure in Huntington's disease patients and controls.** Compared with matched controls resting energy expenditure is significantly higher in Huntington's disease (HD) patients during both basal and insulin stimulated conditions. Bars represent standard errors of the mean. \*  $p < 0.05$ .



### Metabolic parameters in relation to clinical phenotype

When corrected for age and sex, basal and insulin stimulated resting energy expenditure were significantly associated with disease duration ( $r = +0.80$ ,  $p = 0.029$ , and  $r = +0.83$ ,  $p = 0.020$ , respectively), but not with total motor score, chorea, dystonia, rigidity, bradykinesia, total functional capacity or CAG repeat size (all  $p \geq 0.32$ ). However, there was a strong negative association between insulin sensitivity, defined as the glucose disposal rate per kilogram fat free mass during the hyperinsulinemic clamp, and the length of the mutant CAG repeat stretch ( $r = -0.84$ ,  $p = 0.018$ ), while total motor score and total functional capacity were not associated with insulin sensitivity (both  $p \geq 0.83$ ). Although in controls there was a significant association between BMI and insulin sensitivity ( $r = -0.87$ ,  $p = 0.012$ ), this association was reversed and not significant in HD patients ( $r = +0.42$ ,  $p = 0.345$ ).

## DISCUSSION

We present a detailed description of the resting energy expenditure, as well as lipid and glucose metabolism in early stage, medication-free HD patients during both basal and hyperinsulinemic conditions. Compared with matched controls, we found a significantly higher basal resting energy expenditure in HD patients which was primarily due to an increased fat oxidation rate. Moreover, unlike in controls, hyperinsulinemia induced a further increase in energy expenditure in HD patients which was now primarily due to an elevated rate of glucose oxidation. Although we did not find any evidence for insulin resistance in HD patients, higher CAG repeat size was associated with lower insulin sensitivity. These findings point towards defective energy homeostatic mechanisms even in early stage HD patients that could account for the progressive weight loss and muscle wasting in this disorder.

Our finding of a higher resting energy expenditure during basal conditions is in line with a number of previous reports.<sup>27-29</sup> Here we expand on these findings by showing that resting energy expenditure in HD patients increases even further after insulin stimulation, which contrasts with what was found in our controls and what has been reported in other healthy subjects.<sup>30</sup> Insulin is known to stimulate sympathetic nervous system outflow through the ventromedial nucleus of the hypothalamus.<sup>31</sup> Moreover, pathology of this hypothalamic structure is known to impair suppression of sympathetic activity during fasting.<sup>32</sup> Indeed, recently it was shown that HD transgenic mice fail to reduce brown adipose tissue uncoupling protein-1 levels and paradoxically upregulate

PGC-1 $\alpha$  levels during fasting, indicating persistent sympathetic activation.<sup>17</sup> Sympathetic hyperactivity has also been demonstrated in mildly disabled HD patients as well as in otherwise asymptomatic HD mutation carriers.<sup>33</sup> Hence, as sympathetic nervous activity is an important determinant of resting metabolic rate,<sup>31</sup> dysfunction of the ventromedial hypothalamic nucleus in HD may lead to sympathetic hyperactivity and could thereby account for the elevated rate of energy expenditure during both the basal and insulin stimulated state. Paradoxical upregulation of PGC-1 $\alpha$  as a consequence of unabated sympathetic activity may also account for the predominant oxidation of fat during the basal state in HD patients,<sup>17</sup> whereas the relative inability to induce PGC-1 $\alpha$  expression beyond a certain level, as well as enhanced ability of insulin to induce leptin secretion from HD adipocytes may be involved in the increased rate of glucose oxidation during hyperinsulinemic conditions.<sup>15-17,34</sup>

Using the hyperinsulinemic-euglycemic clamp technique, the most accurate method available to assess insulin sensitivity, we could not find any evidence for insulin resistance in early stage HD patients. This result extends findings from recent studies in HD transgenic mouse models and provides conclusive support for the notion that impaired glucose homeostasis in HD is likely due to disturbances of pancreatic insulin release rather than peripheral resistance to insulin.<sup>6-8,35,36</sup> In particular, glucose disposal rate, endogenous glucose production and hepatic insulin sensitivity were all similar between HD patients and controls, both during the basal state and insulin challenge. Although a recent study suggested that insulin resistance may also be involved in HD, this study applied the homeostatic model assessment (HOMA) index for the determination of insulin sensitivity.<sup>10</sup> However, the HOMA index is based on a number of assumptions, such as an intact negative feedback loop between plasma glucose and insulin levels,<sup>37</sup> which may not hold in HD subjects due to, for example, distinct pancreatic islets defects.<sup>6-8</sup> Moreover, the authors did not report on the stage of the illness or medication use in their subjects rendering direct comparisons difficult.<sup>10</sup>

Assessment of the relation between metabolic parameters and clinical characteristics in HD patients revealed a number of interesting associations. Resting energy expenditure during both basal and insulin stimulated states was strongly associated with disease duration, but not with total motor score, chorea, dystonia or functional capacity suggesting a progressive hypermetabolic state in HD patients that is not secondary to increased motor activity or functional impairment. Moreover, higher mutant CAG repeat size was associated with lower insulin sensitivity which could be accounted for by polyglutamine-length dependent interference of mutant huntingtin with both mitochondrial function and transcription of genes that are involved in the insulin signaling pathway.<sup>38,39</sup> However, as our HD cohort as a whole was as sensitive to insulin as the controls, the effect of mutant huntingtin on insulin signaling is either modest or only likely to become appreciable past a certain polyglutamine-tract size; therefore, it would be interesting to evaluate insulin sensitivity in juvenile HD patients who invariably carry very large mutant CAG repeat sizes. The inverse relation between mutant CAG repeat size and insulin sensitivity could also account for the absence of the well-established association between BMI and insulin sensitivity in our HD patients.<sup>40</sup>

In conclusion, we found a higher rate of resting energy expenditure in early stage HD patients that increased further in response to insulin. However, although there was an inverse association between mutant CAG repeat size and insulin sensitivity, HD patients were not insulin resistant. These findings suggest sympathetic hyperactivity as an underlying mechanism of increased energy expenditure in HD, as well as peripheral polyglutamine-length dependent interference of mutant huntingtin with insulin signaling that may become

clinically relevant in carriers of mutations with large CAG repeat sizes.

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**SYNOPSIS,  
CONCLUSIONS  
&  
FUTURE PERSPECTIVES**





*With them the seed of Wisdom did I sow,  
And with mine own hand wrought to make it grow;  
And this was all the Harvest that I reap'd,  
“I came like Water, and like Wind I go.”*

**Omar Khayyam** (1048-1131 CE)

*(Translation from Persian by Edward FitzGerald in 1859)*



## SYNOPSIS & CONCLUSIONS

The nuclear symptoms and signs of Huntington's disease (HD) consist of motor, cognitive and behavioural disturbances. Other less well-known, but prevalent and debilitating features of HD include unintended weight loss, sleep and circadian rhythm disturbances, as well as autonomic nervous system dysfunction. However, the pathogenesis of these less well-known features of HD is poorly understood and currently no effective treatment options are available. It is thus of paramount importance to elucidate the pathological basis of these symptoms and signs in order to design and apply more effective therapeutic interventions. Recently, substantial dysfunction of the hypothalamus was reported in both human studies and various knock-in and transgenic animal models of HD. The hypothalamus consists of groups of interconnected neuronal nuclei located at the base of the brain that regulate a broad array of physiologic, homeostatic and behavioural activities. Therefore, in this thesis we attempt to substantiate the premise that hypothalamic dysfunction per se, as well as secondary (neuro)endocrine and metabolic alterations could contribute to the pathogenesis of several non-motor symptoms and signs of HD (**Chapter 1**).

### *Part I: Secondary signs of HD*

The first part of the thesis is largely devoted to the exploration of the nature and extent of weight loss, sleep and circadian rhythm disturbances, and autonomic complaints in HD patients. In addition, an effort is made to identify the clinical predictors associated with these secondary signs.

A significant decrease in body weight was found over the course of three years in a large group of early stage HD patients (**Chapter 2**). However, no single motor, cognitive or behavioural score was independently associated with weight loss, suggesting that loss of body weight in HD is not secondary to hyperactivity or other symptoms, but likely results from a metabolic defect. As both HD patients and transgenic mice showed a higher rate of weight loss with greater CAG repeat number, this metabolic defect possibly stems directly from polyglutamine length dependent interference of the mutant protein with cellular homeostasis in central (e.g. hypothalamus) or peripheral (e.g. muscle, fat) tissues that are involved in body weight regulation. Moreover, these findings indicate that patients with a higher number of CAG repeats are at an increased risk of unintended weight loss.

The effects of the interaction between mutant and normal *HTT* on clinical phenotype are presented in **Chapter 3**. We found that normal and mutant CAG repeat sizes interact to influence age of onset, and the severity or progression of motor, cognitive and functional symptoms in HD patients, but not body weight. As the effect of the interaction on basal ganglia volume could already be detected in premanifest subjects, these data suggest that the interplay between normal and mutant huntingtin (fragments) directly influences neuronal atrophy or loss and is thus an integral feature of HD pathogenesis. The underlying mechanism may involve interaction of the polyglutamine domains of normal and mutant huntingtin (fragments) and needs further elucidation. These findings may have predictive value and are essential for the design and interpretation of future therapeutic trials.

Data from a systematic evaluation of subjective sleep quality and daytime somnolence in a large cohort of HD patients and premanifest mutation carriers are reported in **Chapter 4**. The findings indicate that while night-time sleep impairment is more prevalent in HD patients, daytime sleepiness appears unlikely to be a major issue in HD. We also show that depression is the most important clinical predictor of sleep impairment in HD patients. Moreover, our findings suggest a delayed sleep phase syndrome-like circadian rhythm disorder in HD patients which appears to be associated with lower cognitive performance.

Findings from a questionnaire study indicated that HD patients experience various symptoms suggestive of autonomic nervous system dysfunction (**Chapter 5**). The complaints particularly concerned the gastrointestinal, urinary, cardiovascular and male sexual domains, and some of them were also present in premanifest mutation carriers. These findings indicate that, contrary to common belief, autonomic symptoms are highly prevalent in HD patients and may even precede the onset of motor signs. Moreover, we found that a greater degree of autonomic dysfunction in HD patients was associated with more functional disability as well as more depressive symptoms, suggesting that adequate management of autonomic symptoms in HD patients could be evaluated as a potential strategy to improve their quality of life.

## ***Part II: Hypothalamic pathology in HD***

In this section (**Chapter 6**) we report a significant reduction by about 30% in the total number of hypocretin-1 immunoreactive neurons in the lateral hypothalamus of HD patients. This decrease appears to be relatively specific as the total number of melanin-concentrating hormone (MCH) immunoreactive neurons was not significantly altered. Hypocretin-1 levels in the prefrontal cortex were reduced to the same extent, but ventricular cerebrospinal fluid levels were unchanged. It remains to be shown whether this moderate decrease in hypocretin signalling could contribute to clinical symptoms. As MCH cell number was not clearly affected in HD patients, alterations in MCH neurotransmission are unlikely to have clinical effects in HD. Interestingly, neuronal intranuclear and cytoplasmic inclusions were not uniformly present in various hypothalamic and adjacent structures in HD patients. This finding may indicate that various hypothalamic nuclei are differentially affected by inclusion formation despite their close anatomical juxtaposition in the hypothalamus.

## ***Part III: Endocrine studies in HD***

A detailed description of cortisol secretory dynamics in patients with HD is presented in **Chapter 7**. We found that the total 24 h cortisol production rates were significantly elevated in HD patients. The increase in cortisol production was primarily confined to the morning and early afternoon period. In addition, circadian rhythmicity analysis revealed a significantly higher amplitude of the diurnal cortisol concentration profile in HD patients. These findings point towards a disturbed central glucocorticoid feedback regulation in HD patients and indicate that hypothalamic-pituitary-adrenal axis dysfunction is an early feature of the disease.

We found no significant differences in growth hormone and ghrelin secretion characteristics between HD patients and controls (**Chapter 8**). However, in HD patients, both growth hormone secretion and its irregularity as well as the degree of postprandial ghrelin suppression significantly increased with worsening motor and functional impairment. Moreover, postprandial ghrelin suppression also increased with decreasing body

weight and higher CAG repeat number. These findings suggest subtle changes in the regulation of growth hormone and ghrelin secretion dynamics in early stage HD patients that may become more pronounced in the later stages of the disease.

Investigation of the thyrotropic and lactotropic axes function (**Chapter 9**) revealed a mild hyperactivity of the hypothalamic-pituitary-thyroid axis, as well as a more irregular pattern of prolactin secretion in HD patients compared with matched controls. These findings are consistent with disrupted hypothalamic-pituitary dopamine signaling in HD. Interestingly, higher free  $T_4$  levels were associated with larger mutant CAG repeat sizes. In addition, there was an inverse trend for the relation between total  $T_4$  levels and body mass index (BMI) in HD patients. As thyroid hormones are known to increase energy expenditure, elevated thyroid hormone levels in early stage HD patients that seem to increase with mutant CAG repeat size, may contribute to the lower BMI in HD mutation carriers,<sup>1,2</sup> and possibly account for the association between mutant *HTT* CAG repeat size and weight loss in HD.<sup>3</sup>

The plasma levels and diurnal rhythmicity of the adipokines leptin, adiponectin and resistin did not significantly differ between HD patients and controls (**Chapter 10**). However, when corrected for fat mass, both mean plasma leptin concentration and secretion rate significantly increased with the size of the CAG repeat mutation in HD patients. As leptin is an anorexigenic hormone that stimulates energy expenditure,<sup>4</sup> enhanced leptin production rate in HD patients with higher CAG repeat lengths in the mutant allele could lead to decreased appetite and hypermetabolism, thereby contributing to the higher rate of weight loss in these subjects.<sup>3</sup> Interestingly, unlike in controls, neither BMI nor body fat mass was significantly related to leptin production in HD patients. These findings suggest that the HD mutation interferes with adipose tissue function, which may contribute to weight loss in HD patients.

The timing of the evening rise in melatonin levels was significantly delayed by more than one and a half hours in early stage HD patients compared with control subjects (**Chapter 11**). Moreover, despite similar mean diurnal melatonin levels between HD patients and controls, we found strong negative associations between mean diurnal melatonin levels and both motor and functional disability in these patients. These findings suggest a delayed sleep phase syndrome-like circadian rhythm disorder in early stage HD patients and suggest that melatonin levels may progressively decline with advancing disease.

#### ***Part IV: Metabolic studies in HD***

Compared with controls, we found a significantly higher basal resting energy expenditure in HD patients which was primarily due to an increased fat oxidation rate (**Chapter 12**). Moreover, unlike in controls, hyperinsulinemia induced a further increase in energy expenditure in HD patients which was now primarily due to an elevated rate of glucose oxidation. Although we did not find any evidence for insulin resistance in HD patients, higher CAG repeat size was associated with lower insulin sensitivity. These findings suggest sympathetic hyperactivity, possibly due to dysfunction of the hypothalamic ventromedial nucleus, as well as peripheral polyglutamine length dependent interference of mutant huntingtin with insulin signaling that may become clinically relevant in carriers of mutations with large CAG repeat sizes.

## FUTURE PERSPECTIVES

The studies described in this thesis provide fertile ground for further basic as well as clinical research into several facets of HD. First, however, alike every other claim of scientific advance, our assertions should be subjected to scrutiny and attempts should be undertaken to replicate the findings in other, preferably larger, cohorts of HD patients. Moreover, it would be interesting to assess patients in various stages of the disease, although medication use as well as functional and mental disabilities in more advanced stage patients posit major feasibility challenges. In this regard, it is particularly encouraging to note that several of our results, such as the inverse association between body weight and mutant CAG repeat size and increased cortisol levels, have already been replicated in independent investigations implicating relatively large numbers of HD patients,<sup>5,6</sup> while confirmational studies are underway for several other of our findings, including the effect of normal and mutant *HTT* interaction on disease severity that will be assessed in the TRACK-HD cohort.<sup>7</sup> With this conditional stipulation in mind, in the remaining part of this section we will put forth a few lines of thought that may serve to direct future research endeavours in this field. The mechanism underlying the negative association between body weight and mutant CAG repeat length needs further elucidation, as this may facilitate the generation of therapeutics aiming to restore cellular energy homeostasis in HD. In particular, it remains to be shown to what extent the relation between body weight and mutant CAG repeat size is due to polyglutamine length dependent pathology of brain structures involved in energy homeostasis (e.g. the hypothalamus),<sup>8,9</sup> or due to pathology of peripheral tissues such as muscle, fat and pancreatic tissue.<sup>10-12</sup> Evaluation of the association between CAG repeat size and the functional integrity of the hypothalamus, for instance as assessed by various imaging techniques, combined with hormone challenge tests and muscle/fat biopsies may greatly advance our understanding of this issue. Likewise, the pathways mediating the effect of the interaction between normal and mutant *HTT* CAG repeat sizes on disease severity await further clarification. Several models could account for this phenomenon, including competitive polyglutamine length dependent interaction of normal and mutant huntingtin with numerous protein binding partners,<sup>13,14</sup> mitochondrial energy production<sup>15</sup> or transcriptional mechanisms.<sup>16</sup> Regardless of the responsible mechanisms, however, an important corollary of this study is that future clinical trials aiming to assess the efficacy of a particular therapy in HD patients should consider adjusting the outcome for the CAG repeat sizes in both *HTT* alleles. Findings from our inventory of sleep disturbances, as well as cortisol and melatonin rhythms in HD indicate a delayed sleep-phase syndrome-like circadian rhythm disturbance that requires further scrutiny by means of other measures of circadian rhythm such as 24 h body temperature recordings and actigraphy. This is important as HD patients may benefit from similar strategies applied for the re-entrainment of the circadian rhythms in subjects suffering from a delayed-sleep phase syndrome. We also found that HD patients experience a large number of symptoms that, at least partly, could be manifestations of autonomic nervous system dysfunction. Application of more extensive, standardized questionnaires in combination with objective measures of autonomic nervous system function may help to elucidate to what degree putative symptoms of autonomic origin actually stem from autonomic failure in HD, rather than being expressions of pathognomonic debilities such as motor impairment. Neuropathological assessment of the hypothalamus revealed that neuronal intranuclear and cytoplasmic inclusions are not uniformly present in various hypothalamic and adjacent structures in HD. Characterization of the processes mediating this heterogeneity may illuminate why certain neuronal populations are more susceptible to HD pathology than others. In this respect, it would be interesting to assess whether Rhes (Ras homologue enriched in striatum), a protein that is thought to mediate the relative selectivity of striatal pathology in HD,<sup>17</sup> is present in the hypothalamus, whether its distribution differs between various hypothalamic nuclei, and whether its expression is altered in HD hypothalami. The findings from our endocrine and metabolic



studies in early stage HD patients are consistent with hypothalamic pathology, and especially implicate the suprachiasmatic nucleus in the disease process. Employing immunocytochemical and *in situ* hybridization techniques, systematic neuropathological evaluation of the suprachiasmatic and other hypothalamic nuclei in HD are currently underway in close collaboration with the Netherlands Institute for Neurosciences. In addition, regarding the progressive nature of HD, longitudinal studies of endocrine (particularly cortisol and growth hormone secretion) and metabolic (especially energy expenditure and response to insulin challenge) parameters in conjunction with clinical assessments may provide more insight into potential dynamic alterations which might prove useful as biomarkers of disease progression. Last but not least, it should be noted that certain of our findings, such as the association between the secretion of the adipocyte specific hormone leptin and mutant CAG repeat size, are better explained by peripheral, rather than central, pathology in HD. Hence, dictated by the expression of mutant huntingtin throughout the body, a holistic approach with the evaluation of both central as well as peripheral tissues appears a prudent strategy for the identification of the pathological basis of secondary signs in HD.<sup>10</sup>

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# ***A**PPENDICES*

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***A.** Summary and conclusions in Dutch*

***B.** Excerpt in Persian*

***C.** Acknowledgments*

***D.** Curriculum vitae*

***E.** List of publications*





## SAMENVATTING & CONCLUSIES

De ziekte van Huntington (ZvH) is een erfelijke neurodegeneratieve aandoening die wordt gekenmerkt door progressieve motorische, psychiatrische en cognitieve stoornissen.<sup>1</sup> Andere minder bekende symptomen van de ZvH, die niettemin frequent voorkomen en de levenskwaliteit nadelig kunnen beïnvloeden, zijn ongewenst gewichtsverlies en stoornissen van de slaap en het autonome zenuwstelsel. Echter, de pathogenese van deze laatstgenoemde symptomen bij de ZvH is grotendeels onopgehelderd en thans zijn bijgevolg geen effectieve therapeutische opties voorhanden. Teneinde het ontwerpen en toepassen van effectieve therapeutische interventies te bespoedigen, is het van groot belang om de pathologische basis van deze ziekteverschijnselen te achterhalen. Recent werden aanwijzingen gevonden voor een aanzienlijke mate van hypothalamische pathologie bij zowel patiënten met de ZvH als muismodellen van de ziekte. De hypothalamus is gelokaliseerd aan de basis van het brein en bestaat uit groepen van onderling verbonden neuronale kernen die tezamen zorgdragen voor de regulatie van een groot aantal fysiologische, homeostatische en adaptieve functies. Ergo, in dit proefschrift hebben wij gepoogd om de premisse te substantiëren dat zowel hypothalamische disfunctie op zich, alsmede secundaire (neuro)endocriene en metabole veranderingen zouden kunnen bijdragen aan de pathogenese van diverse niet-motorische symptomen van de ZvH (**Hoofdstuk 1**).

### *Deel I: Secundaire symptomen van de ZvH*

Het eerste gedeelte van dit proefschrift is grotendeels gewijd aan het verkennen van de aard en de mate van gewichtsverlies en stoornissen in het slaappatroon, het circadiaanse ritme en de functie van het autonome zenuwstelsel bij patiënten met de ZvH. Tevens is getracht om de klinische correlaten van deze secundaire symptomen te identificeren.

In de loop van een drie jaar durend vervolgonderzoek werd een significante afname van het lichaamsgewicht gevonden in een omvangrijke groep patiënten met de ZvH die in een relatief vroeg stadium van de ziekte verkeerden (**Hoofdstuk 2**). Evenwel, geen enkele motorische, cognitieve of gedragsscore was onafhankelijk geassocieerd met de mate van gewichtsverlies, hetgeen erop kan duiden dat gewichtsverlies bij de ZvH niet secundair is aan hyperactiviteit of andere ziekteverschijnselen, maar waarschijnlijk zijn oorsprong vindt in een metabool defect. Aangezien bij zowel patiënten met de ZvH als transgene muizen de snelheid van het gewichtsverlies bleek samen te hangen met het aantal CAG repeat-herhalingen, zou het metabole defect rechtstreeks kunnen voortvloeien uit een polyglutamine-lengte afhankelijke interferentie tussen het mutante huntingtine eiwit en cellulaire homeostase in hetzij centrale (bijv. hypothalamus) of perifere (bijv. spier, vet) weefsels die nauw zijn betrokken bij gewichtsregulatie. Bovendien wijzen deze bevindingen erop dat patiënten met een groter aantal CAG repeat-herhalingen een verhoogd risico hebben op ongewenst gewichtsverlies.

De klinische effecten van de interactie tussen het normale en het mutante *HTT* allel worden gepresenteerd in **Hoofdstuk 3**. De interactie tussen normale en mutante CAG repeatlengtes bleek invloed te hebben op zowel de aanvangsleeftijd van de ZvH als de ernst of progressie van de motorische, cognitieve en functionele symptomen. Echter, psychiatrische symptomen en lichaamsgewicht bleken niet te worden beïnvloed. Aangezien het effect van de interactie op het volume van de basale ganglia tevens reeds in premanifeste mutatie dragers kon worden

gedetecteerd, suggereren deze data dat het samenspel tussen normale en mutante huntingtine (fragmenten) rechtstreeks neuronale atrofie of verlies kan beïnvloeden en dientengevolge een integraal kenmerk van de pathogenese van de ZvH zou kunnen vormen. Het onderliggende mechanisme zou een interactie tussen de polyglutamine domeinen van normale en mutante huntingtine eiwitten kunnen behelzen en behoeft nadere opheldering. Deze bevindingen hebben enige voorspellende waarde en zijn cruciaal voor het ontwerpen en interpreteren van de uitkomsten van toekomstige interventieonderzoeken.

In **Hoofdstuk 4** wordt een systematische evaluatie van de subjectieve slaapkwaliteit alsmede mogelijke klachten van slaperigheid overdag gepresenteerd bij zowel patiënten met de ZvH als premanifeste mutatie dragers. De bevindingen duiden erop dat de slaapkwaliteit vooral 's nachts is verminderd bij de ZvH, terwijl slaperigheid overdag waarschijnlijk weinig voorkomt. Depressie bleek de belangrijkste klinische voorspeller van slaapproblemen te zijn. Bovendien suggereren de resultaten dat de slaapfase bij patiënten met de ZvH naar later op de dag is verschoven en dat deze faseverschuiving samenhangt met een lager niveau van cognitief functioneren.

Bevindingen uit een vragenlijstonderzoek wezen erop dat patiënten met de ZvH last hebben van verschillende symptomen die suggestief zijn voor een stoornis van het autonome zenuwstelsel (**Hoofdstuk 5**). De klachten betroffen voornamelijk de gastro-intestinale, urogenitale en cardiovasculaire domeinen bij patiënten met de ZvH, terwijl enkele klachten tevens aanwezig waren in premanifeste mutatie dragers. De strekking van deze bevindingen is dat, in tegenstelling tot de gangbare notie, autonome klachten vaak voorkomen bij patiënten met de ZvH en zelfs vooraf kunnen gaan aan de motorische manifestatie. Voorts bleek dat de mate van autonome klachten bij patiënten met de ZvH samenhangt met de graad van functionele beperking en depressie, hetgeen erop kan duiden dat adequate behandeling van autonome klachten bij patiënten met de ZvH zou kunnen worden geëvalueerd als een strategie om de levenskwaliteit te verbeteren.

## ***Deel II: Hypothalamie pathologie bij de ZvH***

In deze sectie (**Hoofdstuk 6**) rapporteren we een significante reductie van ongeveer 30% in het totale aantal hypocretine-1 immunoreactieve neuronen in de laterale hypothalamus in post mortem materiaal van patiënten met de ZvH. Deze afname bleek betrekkelijk specifiek aangezien het totale aantal melanine-concentrerend-hormoon (MCH) immunoreactieve neuronen niet significant was veranderd. Hypocretine-1 niveaus in de prefrontale cortex waren eveneens in dezelfde mate afgenomen, maar ventriculaire cerebrospinale vloeistof concentraties waren onveranderd. Het valt te bezien of deze relatief matige afname in hypocretine signaaltransductie klinische consequenties zou kunnen hebben. Aangezien MCH cel aantallen niet duidelijk waren aangetast, is het onwaarschijnlijk dat veranderingen in MCH neurotransmissie klinische repercussies zouden hebben bij de ZvH. Neuronale intranucleaire en cytoplasmatische inclusielichaampjes van mutant huntingtine waren niet uniform aanwezig in de verschillende hypothalamie en belendende structuren, hetgeen zou kunnen inhouden dat de mate van inclusievorming verschilt tussen de verscheidene hypothalamie kernen ondanks hun nauwe anatomische juxtapositie.

## ***Deel III: Endocriene studies bij de ZvH***

Een gedetailleerde beschrijving van de diurnale en secretoire kenmerken van cortisolafgifte is te vinden in **Hoofdstuk 7**. De totale 24-uurs cortisolsecretiesnelheid bleek significant hoger te liggen bij patiënten met de ZvH vergeleken met controlepersonen. De toename in cortisolproductie was primair beperkt tot de ochtend en vroege namiddag. Voorts toonde circadiaanse ritmiciteitsanalyse aan dat de amplitude van het diurnale cortisolprofiel bij patiënten met de ZvH significant hoger was. Deze bevindingen duiden op een ontregelde centrale glucocorticoïd-terugkoppelingsmechanisme en suggereren dat disfunctie van de hypothalamus-hypofyse-bijnieras als een vroeg kenmerk van de ZvH kan worden beschouwd.

De secretiekenmerken van groeihormoon en ghreline bleken niet te verschillen tussen patiënten met de ZvH en controlepersonen (**Hoofdstuk 8**). Evenwel, bij patiënten met de ZvH, bleken zowel de groeihormoonsecretie als diens irregulariteit van afgifte, evenals de postprandiale suppressie van ghrelinesecretie significant toe te nemen met de ziekteprogressie. Daarnaast bleek de postprandiale suppressie van ghrelinesecretie eveneens toe te nemen met een afnemend lichaamsgewicht of een toenemend aantal CAG repeatherhalingen. Deze observaties suggereren subtiele veranderingen in de regulatie van groeihormoon- en ghrelineafgifte in een vroeg stadium van de ZvH die wellicht geprononceerder zouden kunnen worden in de latere stadia van deze aandoening.

Evaluatie van de thyreotrope en lactotrope assen bij patiënten met de ZvH (**Hoofdstuk 9**) liet een milde hyperactiviteit van de hypothalamus-hypofyse-schildklieras zien, evenals een meer irregulair patroon van prolactinesecretie vergeleken met controlepersonen. Deze bevindingen zijn consistent met de notie van een ontregelde hypothalamus/hypofyse dopaminesignaaltransductie bij de ZvH. Hogere vrije  $T_4$  niveaus waren geassocieerd met een groter aantal CAG repeatherhalingen. Voorts waren de totale  $T_4$  niveaus negatief geassocieerd met de 'body-mass-index' (BMI). Aangezien schildklierhormonen het energieverbruik kunnen stimuleren, zouden de verhoogde schildklierhormoonconcentraties bij de ZvH die samenhangen met het aantal CAG repeatherhalingen bij kunnen dragen aan de lagere BMI bij de mutatie dragers,<sup>2,3</sup> en mogelijk tevens ten dele de relatie tussen het aantal mutante *HTT* CAG repeatherhalingen en gewichtsverlies kunnen verklaren.<sup>4</sup>

De plasmaconcentraties en de diurnale ritmiciteit van de adipokinen leptine, adiponectine en resistine verschilden niet significant tussen patiënten met de ZvH en controlepersonen (**Hoofdstuk 10**). Na correctie voor vetmassa bleken nochtans zowel de gemiddelde plasmaconcentratie als de secretiesnelheid van leptine significant toe te nemen met een groter aantal CAG repeatherhalingen bij patiënten met de ZvH. Daar leptine als een anorexinogeen hormoon wordt beschouwd dat het energieverbruik stimuleert,<sup>5</sup> zou toegenomen leptinesecretiesnelheid bij patiënten met een groter aantal CAG repeatherhalingen in het mutante allel tot een verminderde eetlust en hypermetabolisme kunnen leiden en daarmee bijdragen aan de hogere mate van gewichtsverlies bij deze individuen.<sup>4</sup> Het was tevens frappant dat bij patiënten met de ZvH noch de BMI, noch de vetmassa significant waren geassocieerd met de leptineproductie. Deze observaties suggereren dat de mutatie bij de ZvH op enigerlei wijze interfereert met de functie van het vetweefsel.

Ten opzichte van de controlepersonen was het tijdstip van de avondelijke toename van de melatonineconcentratie met ongeveer anderhalf uur verlaat bij patiënten met de ZvH (**Hoofdstuk 11**). Tevens bleek dat, ondanks gelijke diurnale melatonineniveaus tussen patiënten en controles, er sterke negatieve associaties bestonden tussen de gemiddelde diurnale melatonineniveaus en zowel de motorische als de functionele beperkingen bij de patiënten. Deze bevindingen duiden op een 'delayed sleep-phase syndrome'-achtige verstoring van het circadiaanse ritme in de vroege stadia van de ZvH en suggereren dat de melatonineconcentraties mogelijk



progressief zouden kunnen afnemen met het voortschrijden van de ziekte.

#### ***Deel IV: Metabole studies bij de ZvH***

Vergeleken met de controlepersonen was het energieverbruik in rust significant hoger bij patiënten met de ZvH, hetgeen primair werd veroorzaakt door een hogere oxidatie van vet (**Hoofdstuk 12**). Anders dan bij de controlepersonen bleek hyperinsulinemie daarenboven een verdere toename van het energieverbruik te induceren, hetgeen in dit geval voornamelijk door een hogere oxidatie van glucose werd teweeggebracht. Ofschoon er geen aanwijzingen werden gevonden voor insulineresistentie bij patiënten met de ZvH, hing een groter aantal CAG repeatherhalingen in het mutante allel samen met een lagere insulinegevoeligheid. Deze bevindingen wijzen op sympathische hyperactiviteit, mogelijkterwijs ten gevolge van disfunctie van de ventromediale kern van de hypothalamus, alsmede perifere polyglutamine-lengte afhankelijke interferentie van mutant huntingtine met insulinesignaaltransductie die eventueel klinisch relevant zou kunnen zijn in dragers van mutaties met een groot aantal CAG repeatherhalingen.

#### **TOEKOMSTPERSPECTIEVEN**

De studies beschreven in dit proefschrift vormen een vruchtbare grond voor zowel verder basaal als klinisch onderzoek naar verscheidene facetten van de ZvH. Echter, ten eerste behoeven onze beweringen nadere staving middels pogingen om de beschreven bevindingen te repliceren in andere, en bij voorkeur grotere, cohorten patiënten. Voorts zou het interessant zijn om patiënten in verschillende stadia van de ziekte te beoordelen, alhoewel medicatiegebruik evenals functionele en mentale beperkingen in de latere stadia van de ziekte niet gering te schatten obstakels zouden kunnen vormen voor de haalbaarheid van zulk onderzoek. In dit opzicht is het inzonderheid bemoedigend om op te merken dat meerdere van onze bevindingen, zoals de omgekeerde associatie tussen het lichaamsgewicht en de grootte van de mutante CAG repeatsequentie alsmede verhoogde cortisolconcentraties,<sup>6,7</sup> reeds zijn gerepliceerd in onafhankelijke studies met betrekkelijk grote aantallen patiënten, terwijl confirmerende studies onderweg zijn voor andere bevindingen zoals het klinische effect van de interactie tussen het normale en het mutante *HTT* die nader zal worden beoordeeld in de TRACK-HD cohort.<sup>8</sup> Met deze voorwaardelijke stipulatie in het achterhoofd, zullen wij in het resterende deel van deze sectie een klein aantal gedachtegangen entameren die mogelijk richting zouden kunnen geven aan verdere onderzoeksinspanningen in dit veld. Het verantwoordelijke mechanisme voor de negatieve associatie tussen het lichaamsgewicht en de mutante CAG repeatlengte behoeft nadere opheldering aangezien zodoende het ontwikkelen van nieuwe behandelingen die herstel van de cellulaire energiehomeostase bij de ZvH beogen, zou kunnen worden gefaciliteerd. In het bijzonder dient te worden beoordeeld in hoeverre de associatie tussen het lichaamsgewicht en het aantal CAG repeatherhalingen het gevolg is van polyglutamine-lengte afhankelijke pathologie van hersencentra betrokken bij de energiehomeostase (bijv. de hypothalamus),<sup>9,10</sup> of toe te schrijven is aan defecten in perifere weefsels zoals spier- en vetweefsel.<sup>11-13</sup> Evaluatie van de associatie tussen CAG repeatlengte en de functionele integriteit van de hypothalamus, bijvoorbeeld zoals beoordeeld middels verschillende beeldvormende technieken in combinatie met hormonale functietesten en spier-/vetbiopsieën, zou ons begrip inzake aanzienlijk kunnen verbeteren. Evenzo behoeven de mechanismen die het effect van de interactie tussen normale en mutante CAG repeatlengtes mediëren nadere verduidelijking. Diverse modellen zouden dit fenomeen kunnen verklaren, zoals een polyglutamine-lengte afhankelijke interactie

tussen het normale en het mutante huntingtine eiwit met onder meer een groot scala aan bindingspartners,<sup>14,15</sup> mitochondriale energieproductie<sup>16</sup> of transcriptieregulatie.<sup>17</sup> Niettemin, ongeacht het verantwoordelijke mechanisme, is een belangrijk uitvloeisel van onze bevindingen dat in toekomstige klinische experimentele onderzoeken met het oogmerk om de potentiële merites van een specifieke behandeling bij de ZvH te beoordelen de uitkomsten dienen te worden gecorrigeerd voor de CAG repeatlengtes in beide *HTT* allelen. De resultaten van de inventarisatie van de slaapproblemen, evenals de analyses van de diurnale secretiepatronen van cortisol en melatonine wijzen op een ‘delayed sleep-phase syndrome’-achtige circadiaanse ritmestoornis die nader zou kunnen worden gekarakteriseerd middels andere maten voor het beoordelen van het circadiaanse ritme zoals 24-uurs lichaamstemperatuurmetingen en actigrafie. Dit is belangrijk omdat patiënten met de ZvH eventueel zouden kunnen profiteren van maatregelen bedoeld voor het herinstellen van het circadiaanse ritme. Patiënten met de ZvH bleken tevens last te hebben van een groot aantal klachten die, tenminste gedeeltelijk, manifestaties zouden kunnen zijn van disfunctie van het autonome zenuwstelsel. Het aanwenden van extensievere standaardvragenlijsten in combinatie met objectieve maten voor het functioneren van het autonome zenuwstelsel zouden kunnen helpen om te verhelderen in hoeverre vermeende symptomen van autonome origine daadwerkelijk ontspruiten aan autonoom falen, ofwel manifestaties zijn van pathognomonische verschijnselen zoals motorische stoornissen. Neuropathologische evaluatie van de hypothalamus wees uit dat neuronale intranucleaire en cytoplasmatische inclusielichaampjes van mutant huntingtine niet uniform aanwezig zijn in de verschillende hypothalamische en belendende structuren bij de ZvH. Karakterisatie van de processen die deze heterogeniteit teweegbrengen, zou kunnen verhelderen waarom bepaalde neuronale populaties gevoeliger zijn dan andere voor de pathologie van de ZvH. In dit opzicht zou het interessant zijn om te beoordelen of Rhes (‘Ras homologue enriched in striatum’), een eiwit waarvan wordt vermoed dat het de relatief selectieve striatale pathologie van de ZvH medieert,<sup>18</sup> eveneens aanwezig is in de hypothalamus, of de distributie ervan verschilt tussen de diverse hypothalamische kernen, en of de expressie ervan is veranderd in de hypothalami van patiënten met de ZvH. De uitkomsten van de endocriene en metabole experimenten bij patiënten met de ZvH in een vroeg stadium van de aandoening zijn consistent met de notie van hypothalamische pathologie en wijzen inzonderheid op de betrokkenheid van de suprachiasmatische kern bij het ziekteproces. Gebruikmakend van immunocytochemische en *in situ* hybridisatie technieken zijn thans systematische neuropathologische studies van de suprachiasmatische en andere hypothalamische kernen onderweg in nauwe samenwerking met het Nederlands Instituut voor Neurowetenschappen. Gezien het progressieve karakter van de ZvH zouden voorts longitudinale studies van endocriene (in het bijzonder de cortisol- en groeihormoonsecretie) en metabole (met name het energieverbruik en de respons op insulinstimulatie) parameters in samenhang met klinische evaluaties meer inzicht kunnen verschaffen in potentiële dynamische veranderingen die eventueel bruikbaar zouden kunnen zijn als biomarkers voor de ziekteprogressie. En ten slotte zij opgemerkt dat enkele van onze bevindingen, zoals de associatie tussen de secretie van het adipocytenhormoon leptine en mutante CAG repeatlengte, veeleer kunnen worden begrepen in het licht van perifere dan centrale pathologie bij de ZvH. Ergo, gedicteerd door het feit dat mutant huntingtine bijna overal in het lichaam tot expressie wordt gebracht, schijnt een holistische benadering met zowel oog voor de centrale als de perifere weefsels een prudente strategie voor het achterhalen van het pathologische substraat van de secundaire symptomen bij de ZvH.<sup>13</sup>

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یک چند به کودکی به استاد شدیم  
یک چند به استادی خود شاد شدیم  
پایان سخن شنو که ما را چه رسید  
از آب بر آمدیم و بر باد شدیم

عمر خیام (۵۱۰-۴۲۷ شمسی)



### خلاصه

بیماری هانتینگتون یک بیماری ارثی و انحطاطی نورولوژیکی است که با وخامت تصاعدی علائمی چون اختلالات حرکتی، فکری و روانی همراه می‌باشد. علائم کمتر شناخته شده دیگر بیماری هانتینگتون که مرتب نزد این بیماران دیده شده و تاثير منفی بر کیفیت زندگی آنها می‌گذارند، عبارتند از: از دست دادن وزن به صورت ناخواسته، اختلالات خواب و سیستم عصبی نباتی.

در حقیقت بیماریزایی این علائم کمتر شناخته شده فوق نزد بیماران هانتینگتون هنوز تماماً روشن نشده و بهمین دلیل تا کنون هم درمان موثری برای آنها کشف نگردیده است. بنابراین برای تسریع بخشیدن به طرح و اعمال روشهای موثر درمانی، روشن شدن پاتوژنز این علائم از اهمیت بسیار زیادی برخوردار می‌باشد. اخیراً شواهدی مبنی بر آسیبهای مشخص در هیپوتالاموس بیماران هانتینگتون و نمونه های حیوانی مبتلا به این بیماری پیدا شده است. هیپوتالاموس خود در قاعده مغز قرار گرفته و متشکل شده از گروه های نورونی بهم پیوسته بوده که با همدیگر مسئول تنظیم تعداد زیادی از مکانیزمهای فیزیولوژیکی و وفقی بدن می‌باشند.

در این رساله دکتری کوشش میکنیم که با دلیل و مدرک اثبات کنیم که آسیب هیپو تا لاموس و همچنان تغییرات ثانوی در کار غدد درون ریز و متابولیسم بدن میتوانند باعث بیماریزایی تعدادی از علائم غیرحرکی بیماری هانتینگتون شوند.





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## **CURRICULUM VITAE**

On December 7, 1981 Nasir Ahmad Aziz was born in Kandahar (Afghanistan). He came to the Netherlands in 1994, and the next year he started secondary education at the ‘Rijnlands Lyceum’ in Sassenheim, obtaining his “VWO” diploma summa cum laude in 2001. During this period he developed a keen interest in mathematics, physics and computer programming which has never left him since. In 2001, he commenced his medical training at the Leiden University Medical Centre (LUMC), where he passed both his “propedaeuse” (2002) and “doctoraal” (2006) examinations cum laude. Following the course “Huntington’s disease” (Prof. Dr. R.A.C. Roos) in the second year of the curriculum, he became involved in several research activities on this disorder as part of the LUMC’s Excellent Students’ Program, culminating in the dissertation at hand. He was also selected for the LUMC’s Honours Program (2005), received a Mosaic prize from the Netherlands Organization for Scientific Research for the research project “Neuroendocrine and metabolic abnormalities in Parkinson’s and Huntington’s disease in relation to clinical presentation” (2006), and was a co-applicant of an awarded grant by the CHDI/High Q foundation for the research proposal “The hypothalamus in patients with Huntington’s disease” (2008). The PhD work was performed at the LUMC’s Departments of Neurology (Prof. Dr. R.A.C. Roos), Endocrinology (Prof. Dr. H. Pijl and Dr. F. Roelfsema), and Clinical Chemistry (Dr. M. Frölich), as well as at the Netherlands Institute for Neuroscience (Prof. Dr. D.F. Swaab). Parts of this research were awarded by the European Huntington’s Disease Network and the World Federation of Neurology. He has been an active member of the students’ debating society “De Leidsche Beck” (now the “Leiden Debating Union”) and the Students’ Association for International Affairs (“SIB Leiden”). In October 2009, he started his clinical internships.



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2 Editorial in *Neurology*; 2009,73(16):1254-5. Comment in: Faculty of 1000 Medicine, <http://www.fl000medicine.com/article/id/1166378/evaluation>. Selected as the article of the month by the European Huntington's Disease Network for the October 2009 issue of the newsletter.





