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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±0.14 vs. 0.91±0.19 mU/L, p=0.365; prolactin: 4.91±0.42 vs. 4.83±0.26 µg/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±0.05 vs. 1.35±0.09, p=0.027; T<sub>4</sub>: 91.9±3.9 vs. 81.3±3.1, p=0.047), while prolactin secretion was significantly more irregular (ApEn ratios: 0.61±0.04 vs. 0.48±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.  $4.83 \pm 0.26$   $\mu\text{g/L}$ ,  $p = 0.872$ ; **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  $\mu\text{g/L/24 h}$  for prolactin.

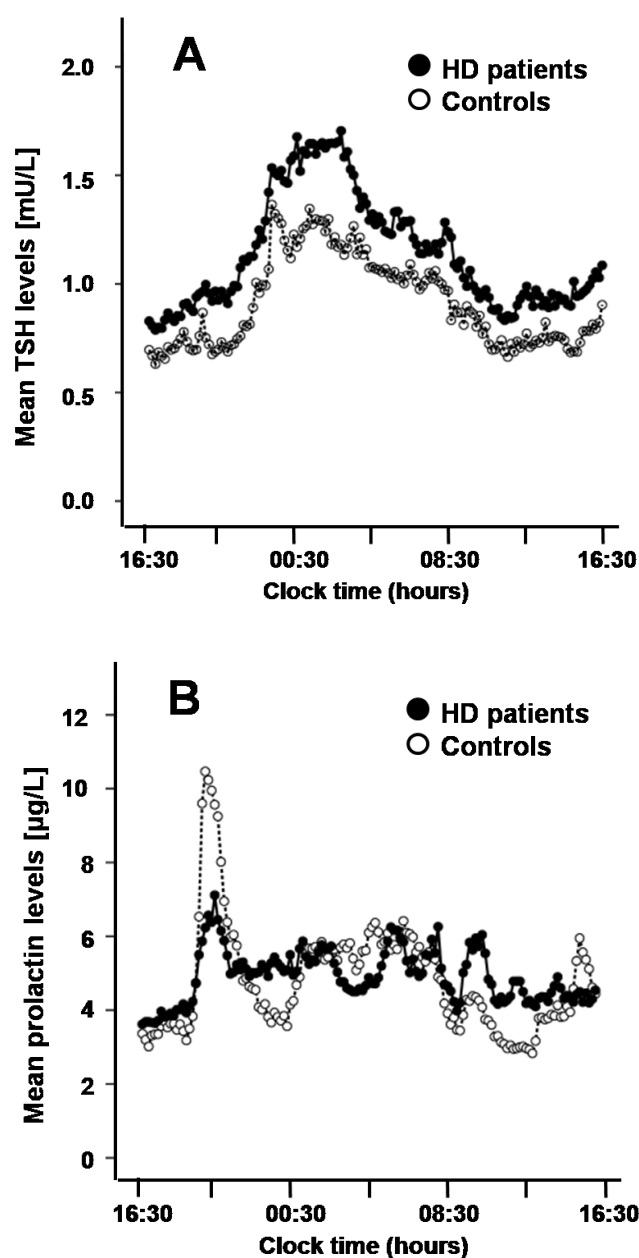
<sup>3</sup>) Mean mass secreted per pulse is in mU/L for TSH, and  $\mu\text{g/L}$  for prolactin.

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

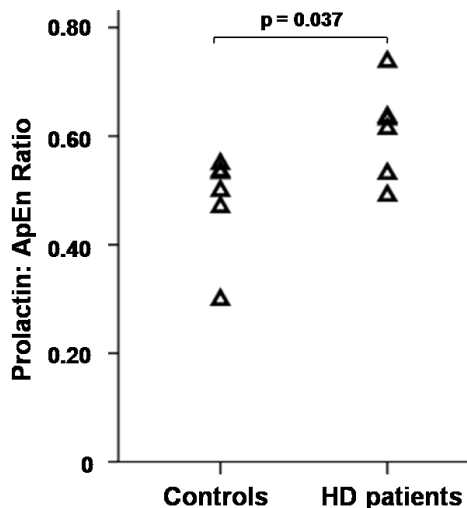
In HD patients, total daily TSH and prolactin secretion rates were not significantly associated with BMI, motor score, total functional capacity or mutant CAG repeat size (all  $p \geq 0.17$ ). However, higher total  $T_3$  levels were significantly associated with less motor impairment ( $r = -0.72$ ,  $p = 0.030$ ). 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.



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<sub>2</sub> receptor loss. The modest decrease of hypothalamic D<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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

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<sub>2</sub> 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 <sup>11</sup>C-raclopride, a specific D<sub>2</sub> receptor ligand, Politis et al.<sup>27</sup> recently demonstrated a loss of D<sub>2</sub> 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<sub>2</sub> receptors has also been

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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