Huntington's disease : hypothalamic, endocrine and metabolic aspects

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Chapter 10

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.[1] It is characterized by motor disturbances, cognitive decline and behavioral problems.[1] Unintended weight loss is also a hallmark of the disease, both in HD patients [2-5] and several mouse models of the disease.[6,7] Weight loss frequently leads to general weakening and a decline in the quality of life of HD patients.[8] On the other hand, a higher Body Mass Index (BMI) has been associated with a slower rate of disease progression.[9] 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.[2,3]

Interestingly, weight loss in HD patients is accompanied by substantial loss of body fat stores.[4,10,11] Moreover, abnormal fat cell function has been reported in several mouse models of HD.[6,12,13] 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[14], defects in fat metabolism may partly contribute to weight loss in HD patients.[6] 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.[15-19] 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[20]; particularly in light of recent reports of substantial circadian rhythm disturbances in HD[21], 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.[3]

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

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

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 ºC for 20 min, and plasma was stored at -80 ºC until assay. Three standardized meals were served at 0900, 1300, and 1900 h (Nutridrink, 1.5 kcal/ml, 1500–1800 kcal/d; macronutrient composition per 100 ml: protein, 5 g; fat, 6.5 g; carbohydrate, 17.9 g; Nutricia, Zoetermeer, The Netherlands). 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.[23] 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.[23] Apart from the initial concentration and the basal secretion rate, which both were initialized to zero, the *AutoDecon* algorithm requires only two approximations of the parameter values that are to be estimated: (1) The standard deviation of the Gaussian-shaped secretion events (Secretion*SD*) which is generally initialized as half of the data-sampling interval, and (2) and an estimate of the elimination parameter, or hormone half-life.[23] Thus, for 20-min sampled data, the Secretion*SD* 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 ± 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

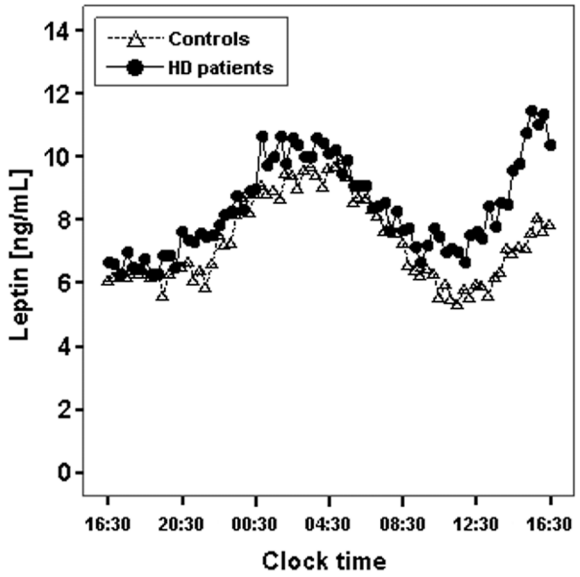
The HD and the control group did not differ with respect to age, sex, BMI, body fat percentage, or lean body mass (all $p \geq 0.15$, Table 1). There were also no significant differences in urinary creatinine, epinephrine, norepinephrine and dopamine levels (all $p \geq 0.10$).

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 ± 3.1 *vs.* 7.4 ± 3.2

µg/L, p = 0.823). Basal, pulsatile and total leptin secretion rates were also not significantly different between the two groups (all p ≥ 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

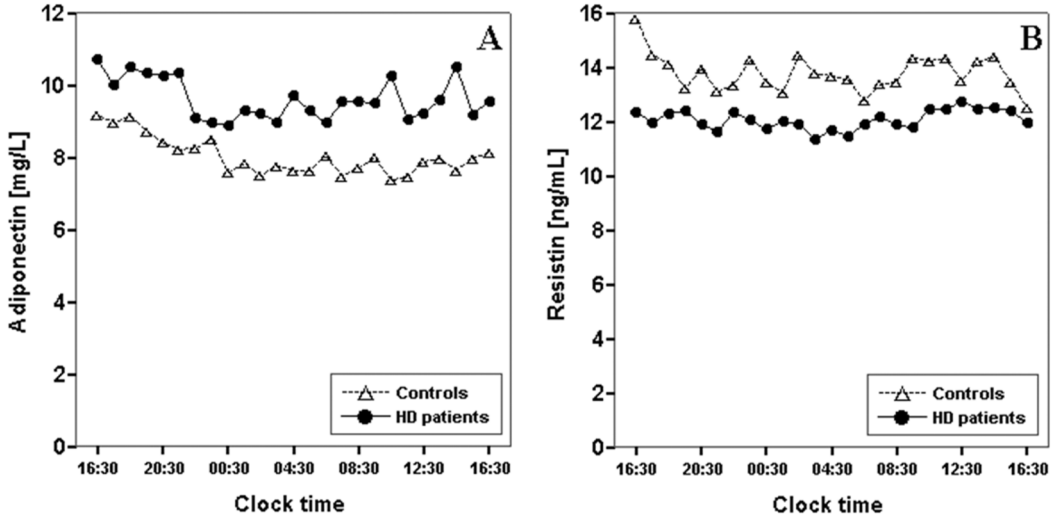
The average 24 h plasma adiponectin and resistin concentration profiles of HD patients and controls are shown in Figure 2. Although throughout the circadian cycle adiponectin levels were higher and resistin levels were lower in HD patients compared with controls, the two groups did not differ significantly with respect to mean 24 h levels of adiponectin (9.6 ± 1.9 *vs.* 8.0 ± 1.6 mg/L, p = 0.540) or resistin (12.0 ± 1.7 *vs.* 13.8 ± 1.6 µg/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†	Controls†	p-value‡
Basal secretion rate [µg/L/24 h]	0.08 (0.03)	0.07 (0.03)	0.791
Pulsatile secretion rate [µg/L/24 h]	35 (12)	30 (13)	0.785
Total secretion rate [µg/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 [µg/L]	5.0 (1.6)	5.0 (2.1)	0.992

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

†) 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.[14] 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.[24,25] 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.[17] 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[6], 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.[3] Similarly, R6/2 mice with larger mutant CAG repeat lengths in the transgene had a lower body weight.[3] Here we show that the production rate of leptin, an anorexigenic hormone that stimulates energy expenditure[14], 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).[26] In HD, gene microarray studies have indicated selective transcriptional alterations of many genes that contain binding sites for Sp1[27,28], which has been shown to interact with huntingtin in a polyglutamine length-dependent manner.[29] 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.[30] 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[27], 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 [16,17], others reported no change.[15,18,19] 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.[24] 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[14], 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 tract 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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REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72(6):971-983.

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

3. Aziz NA, van der Burg JM, Landwehrmeyer GB, Brundin P, Stijnen T, EHDI Study Group et al. Weight loss in Huntington disease increases with higher CAG repeat number. Neurology 2008; 71(19):1506-1513.

4. Trejo A, Tarrats RM, Alonso ME, Boll MC, Ochoa A, Velasquez L. Assessment of the nutrition status of patients with Huntington's disease. Nutrition 2004; 20(2):192-196.

5. Djousse L, Knowlton B, Cupples LA, Marder K, Shoulson I, Myers RH. Weight loss in early stage of Huntington's disease. Neurology 2002; 59(9):1325-1330.

6. Phan J, Hickey MA, Zhang P, Chesselet MF, Reue K. Adipose tissue dysfunction tracks disease progression in two Huntington's disease mouse models. Hum Mol Genet 2009; 18(6):1006-1016.

7. van der Burg JM, Bacos K, Wood NI, Lindqvist A, Wierup N, Woodman B et al. Increased metabolism in the R6/2 mouse model of Huntington's disease. Neurobiol Dis 2008; 29(1):41-51.

8. Nance MA, Sanders G. Characteristics of individuals with Huntington disease in long-term care. Mov Disord 1996; 11(5):542-548.

9. Myers RH, Sax DS, Koroshetz WJ, Mastromauro C, Cupples LA, Kiely DK et al. Factors associated with slow progression in Huntington's disease. Arch Neurol 1991; 48(8):800-804.

10. Farrer LA, Meaney FJ. An anthropometric assessment of Huntington's disease patients and families. Am J Phys Anthropol 1985; 67(3):185-194.

11. Farrer LA, Yu PL. Anthropometric discrimination among affected, at-risk, and not-at-risk individuals in families with Huntington disease. Am J Med Genet 1985; 21(2):307-316.

12. Fain JN, Del Mar NA, Meade CA, Reiner A, Goldowitz D. Abnormalities in the functioning of adipocytes from R6/2 mice that are transgenic for the Huntington's disease mutation. Hum Mol Genet 2001; 10(2):145-152.

13. Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER et al. Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab 2006; 4(5):349-362.

14. Trujillo ME, Scherer PE. Adipose tissue-derived factors: impact on health and disease. Endocr Rev 2006; 27(7):762-778.

15. Gaba AM, Zhang K, Marder K, Moskowitz CB, Werner P, Boozer CN. Energy balance in early-stage Huntington disease. Am J Clin Nutr 2005; 81(6):1335-1341.

16. Mochel F, Charles P, Seguin F, Barritault J, Coussieu C, Perin L et al. Early energy deficit in Huntington disease: identification of a plasma biomarker traceable during disease progression. PLoS ONE 2007; 2(7):e647.

17. Popovic V, Svetel M, Djurovic M, Petrovic S, Doknic M, Pekic S et al. Circulating and cerebrospinal fluid ghrelin and leptin: potential role in altered body weight in Huntington's disease. Eur J Endocrinol 2004; 151(4):451-455.

18. Pratley RE, Salbe AD, Ravussin E, Caviness JN. Higher sedentary energy expenditure in patients with Huntington's disease. Ann Neurol 2000; 47(1):64-70.

19. Goodman AO, Murgatroyd PR, Medina-Gomez G, Wood NI, Finer N, Vidal-Puig AJ et al. The metabolic profile of early Huntington's disease--a combined human and transgenic mouse study. Exp Neurol 2008; 210(2):691-698.

20. Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med 1997; 3(5):575-579.

21. Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J Neurosci 2005; 25(1):157-163.

22. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. Mov Disord 1996; 11(2):136-142.

23. Johnson ML, Pipes L, Veldhuis PP, Farhy LS, Boyd DG, Evans WS. AutoDecon, a deconvolution algorithm for identification and characterization of luteinizing hormone secretory bursts: description and validation using synthetic data. Anal Biochem 2008; 381(1):8-17.

24. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334(5):292-295.

25. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med 1995; 1(9):950-953.

26. Gong DW, Bi S, Pratley RE, Weintraub BD. Genomic structure and promoter analysis of the human obese gene. J Biol Chem 1996; 271(8):3971-3974.

27. Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G et al. Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 2006; 15(6):965-977.

28. Luthi-Carter R, Hanson SA, Strand AD, Bergstrom DA, Chun W, Peters NL et al. Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum Mol Genet 2002; 11(17):1911-1926.

29. Dunah AW, Jeong H, Griffin A, Kim YM, Standaert DG, Hersch SM et al. Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 2002; 296(5576):2238-2243.

30. Qiu Z, Norflus F, Singh B, Swindell MK, Buzescu R, Bejarano M et al. Sp1 is up-regulated in cellular and transgenic models of Huntington disease, and its reduction is neuroprotective. J Biol Chem 2006; 281(24):16672-16680.

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

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

†) Values are indicated as mean (SE).

‡) Differences between groups were assessed by unpaired t-tests.