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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 ([6,6-²H₂]-glucose and [²H₅]- 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±72 vs. 1883±93kCal/24 h, p=0.037) and increased even further after insulin stimulation (1667±87 vs. 2068±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.

INTRODUCTION

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.¹ The disease is characterized by motor disturbances, cognitive deterioration, and psychiatric and behavioural problems.² Progressive weight loss and muscle wasting, despite sustained or even increased caloric intake, are also hallmarks of the disease, both in HD patients³⁻⁵ and several transgenic mouse models of the disease.⁶⁻⁹ 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.^{7,10,11} These peripheral abnormalities may not only considerably impair the quality of life of HD patients but could also affect the neurodegenerative process.^{12,13} 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.^{3,13,14}

Several studies have implicated transcriptional repression of the peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) by mutant huntingtin in HD associated neurodegeneration.¹⁵⁻¹⁷ The transcriptional coactivator PGC-1 α 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.¹⁸ Interestingly, recently it was shown that mutant huntingtin also inhibits transcriptional activity of PGC-1 α and its target genes in peripheral tissues such as muscle and fat in both HD patients and animal models of the disease.^{19,20} As PGC-1 α has emerged as a master regulator of systemic energy homeostasis involved in adaptive thermogenesis, β -oxidation of fatty acids, insulin sensitivity and carbohydrate metabolism in general,²¹ we hypothesized that impaired peripheral PGC-1 α 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.²² 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), excessive alcohol intake (>4 units per day), and any other chronic condition except HD as assessed by clinical examination and routine laboratory tests. Although smoking was not an exclusion criterion, all patients and controls were matched for smoking status (only one HD patient and his matched control were smokers). 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

All studies started at 8:00 AM after an overnight fast. Alcohol consumption on the day preceding the study was also not allowed, while on the day of the study, smoking was prohibited until the end of the measurements. 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.²³ 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 [6,6-²H₂]-glucose and [²H₅]- 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 [6,6-²H₂]-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 [²H₅]-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.²⁴ 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 [6,6-²H₂]-glucose – and [²H₅]-glycerol –specific activities. Subsequently, a primed continuous infusion of insulin (Actrapid, Novo Nordisk Pharma BV, Alphen aan de Rijn, The Netherlands; 40 mU/m²/min) was started (t = 120 min). Exogenous glucose 20% enriched with 3% [6,6-²H₂]-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 [6,6-²H₂]-glucose – and [²H₅]-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, [6,6-²H₂]-glucose, and [²H₅]-glycerol were determined in a single analytical run using gas chromatography-mass spectrometry as described previously.²³

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.²⁵ 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.²⁶ 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.²⁴ 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).

Table 1. Characteristics of the study population

| | HD patients[†] | Controls[†] | p-value[‡] |
|-------------------------------|--------------------------------|-----------------------------|----------------------------|
| 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) | - | - |

[†]) 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.

Moreover, fasting levels of glucose, insulin, glycosylated hemoglobin (HbA_{1c}), 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 ($\mu\text{mol}/\text{kg}_{\text{FFM}}/\text{min}$) | 20.7 ± 0.8 | 21.2 ± 0.6 | 0.381 | 65.0 ± 7.2 | 68.6 ± 9.2 | 0.884 |
| Glucose Oxidation ($\mu\text{mol}/\text{min}$) | 653 ± 137 | 709 ± 102 | 0.748 | 1783 ± 227 | 1314 ± 68 | 0.065 |
| NOGD ($\mu\text{mol}/\text{kg}_{\text{FFM}}/\text{min}$) | 12.7 ± 4.3 | 7.6 ± 1.7 | 0.290 | 33.6 ± 6.9 | 40.0 ± 7.8 | 0.543 |
| EGP ($\mu\text{mol}/\text{kg}_{\text{FFM}}/\text{min}$) | 20.7 ± 0.8 | 21.2 ± 0.6 | 0.381 | 13.7 ± 1.0 | 15.0 ± 1.6 | 0.850 |
| HIR ($\mu\text{mol kg}_{\text{FFM}}/\text{min}/\text{pmol} \times \text{L}$) | 1176 ± 126 | 1232 ± 83 | 0.547 | 7841 ± 549 | 7891 ± 595 | 0.558 |
| Glycerol R_a ($\mu\text{mol}/\text{kg}_{\text{FM}}/\text{min}$) | 7.4 ± 0.9 | 6.4 ± 0.8 | 0.409 | 2.6 ± 0.4 | 2.3 ± 0.3 | 0.503 |
| Lipid Oxidation ($\mu\text{mol}/\text{min}$) | 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_I ($\text{ml}/\text{m}^2/\text{min}$) | - | - | - | 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 |
| HbA _{1c} (%) | 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. * $p < 0.05$

EGP = endogenous glucose production; FFM = fat free mass; FM = fat mass; HIR = hepatic insulin resistance; MCR_I = 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 $\mu\text{mol}/\text{min}$, $p = 0.032$).

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).

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.²⁷⁻²⁹ 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.³⁰ Insulin is known to stimulate sympathetic

nervous system outflow through the ventromedial nucleus of the hypothalamus.³¹ Moreover, pathology of this hypothalamic structure is known to impair suppression of sympathetic activity during fasting.³² Indeed, recently it was shown that HD transgenic mice fail to reduce brown adipose tissue uncoupling protein-1 levels and paradoxically upregulate PGC-1 α levels during fasting, indicating persistent sympathetic activation.¹⁷ Sympathetic hyperactivity has also been demonstrated in mildly disabled HD patients as well as in otherwise asymptomatic HD mutation carriers.³³ Hence, as sympathetic nervous activity is an important determinant of resting metabolic rate,³¹ 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 α as a consequence of unabated sympathetic activity may also account for the predominant oxidation of fat during the basal state in HD patients,¹⁷ whereas the relative inability to induce PGC-1 α 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.^{15-17,34} However, as we have not measured sympathetic function in this study, at this stage the contribution of sympathetic dysfunction to metabolic defects in HD remains hypothetical. Therefore, further studies that directly assess sympathetic activity, such as heart rate variability and sympathetic skin response tests, in conjunction with metabolic measures are necessary to define the actual contribution of sympathetic dysfunction to defects in systemic energy homeostasis in HD.

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.^{6-8,35,36} 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.¹⁰ However, the HOMA index is based on a number of assumptions, such as an intact negative feedback loop between plasma glucose and insulin levels,³⁷ which may not hold in HD subjects due to, for example, distinct pancreatic islets defects.⁶⁻⁸ Moreover, the authors did not report on the stage of the illness or medication use in their subjects rendering direct comparisons difficult.¹⁰

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.^{38,39} 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.⁴⁰

One potential limitation of this study is the relatively small number of participants. However, this limitation is largely offset by the application of very sensitive measures of metabolic activity that would have been unfeasible in larger groups of subjects due to the expensive and laborious nature of the

experiments. Hence, although inclusion of larger groups of subjects would have yielded more precise results, it is unlikely that the overall outcome would have been different.

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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Figure legends:

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. * $p < 0.05$.

