Homeostatic adaptations in brain energy metabolism in mouse models of Huntington disease

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Impairment of energy metabolism is a key feature of Huntington disease (HD). Recently, we reported longitudinal neurochemical changes in R6/2 mice measured by *in-vivo* proton magnetic resonance spectroscopy (¹H MRS; Zacharoff *et al*, 2012). Here, we present similar ¹H MRS measurements at an early stage in the milder Q111 mouse model. In addition, we measured the concentration of ATP and inorganic phosphate (P_i), key energy metabolites not accessible with ¹H MRS, using ³¹P MRS both in Q111 and in R6/2 mice. Significant changes in striatal creatine and phosphocreatine were observed in Q111 mice at 6 weeks relative to control, and these changes were largely reversed at 13 weeks. No significant change was detected in ATP concentration, in either HD mouse, compared with control. Calculated values of [ADP], phosphorylation potential, relative rate of ATP synthase ($v/V_{\max}(\text{ATP})$), and relative rate of creatine kinase ($v/V_{\max}(\text{CK})$) were calculated from the measured data. ADP concentration and $v/V_{\max}(\text{ATP})$ were increased in Q111 mice at 6 weeks, and returned close to normal at 13 weeks. In contrast, these parameters were normal in R6/2 mice. These results suggest that early changes in brain energy metabolism are followed by compensatory shifts to maintain energetic homeostasis from early ages through manifest disease.

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Introduction

Alterations in energy metabolism accompany Huntington disease (HD). Brain glucose utilization is reduced (Powers *et al*, 2007), and binding of mutant huntingtin (muhtt) to mitochondrial membranes depresses cellular respiration (Gellerich *et al*, 2008; Orr *et al*, 2008). Decreased expression levels of the iron-sulfur containing subunit of succinate dehydrogenase are evident in postmortem striatum of HD patients at stages 1 to 3, suggesting that decreased ATP production may be an early pathogenic event (Benchoua *et al*, 2006). Indeed, the ratio of ATP

to ADP decreases proportionally to the number of CAG repeats in transformed STHdhQ111 striatal cells and in patient-derived lymphoblast cell lines (Seong *et al*, 2005). Addition of creatine as an energy supplement reduces muhtt aggregates in organotypic slice cultures (Smith *et al*, 2001).

In addition to impaired energy production, there is also evidence pointing to increased energy demand in HD. Accumulation of mitochondria around inclusion bodies suggests that activation of the ubiquitin-proteasomal system in the clearance of mutant htt (muhtt) requires additional ATP generation (Waelter *et al*, 2001). The increased burden associated with synthesis, (mis)-folding, and clearance of muhtt and its binding partners likely adds energy costs (Kelly and Balch, 2006). Oxidative stress, associated with development of the Huntington phenotype, activates PARP, resulting in NADH cycling and ATP depletion (Altmann *et al*, 2006). Increased demand could result from increased signaling and maintenance of ionic gradients accompanying electrophysiological changes (Cepeda *et al*, 2010). Elevation of NMDA receptor stimulated influx of intracellular calcium has also been postulated to increase cellular metabolic load (Fernandes *et al*, 2007).

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Magnetic resonance spectroscopy (MRS) allows noninvasive measurements of the concentration of multiple brain metabolites, several of which are involved in brain energy metabolism. Recently, we reported that proton MRS (^1H MRS) measurements of *in-vivo* neurochemical profiles together with principal component analysis could distinguish R6/2 mice from wild-type (WT) littermates at an earlier age than measurements of changes in brain volume (Zacharoff *et al*, 2012). The R6/2 mouse, however, is considered a severe model, and may not adequately reflect the slow neurodegeneration seen in HD patients. In contrast, the knock-in Q111 mouse exhibits much slower disease onset with few, if any symptoms until the age of 100 weeks (Menalled *et al*, 2009). Since muhtt alters the mitochondrial biogenesis regulator, PGC1 α , examination of brain metabolism before and after nuclear muhtt accumulation may provide more relevant information about changes in brain metabolism in early HD than measurements in manifest disease in R6/2 mice. In Q111 mice, alterations in adenine nucleotide levels, appearance of dark nuclear muhtt staining, and a decreased sensitivity of mitochondria to Ca^{2+} loading have been temporally documented to occur between ~6 and 12 weeks of age (Brustovetsky *et al*, 2005; Gines *et al*, 2003; Wheeler *et al*, 2000).

The aim of the present study was to investigate changes in brain metabolism in Q111 mice at ages (6 and 13 weeks) bracketing these early biochemical events. Specifically, we measured neurochemical profiles in the striatum of Q111 mice at 6 and 13 weeks using ^1H MRS. Similar ^1H MRS data were already reported in the cortex and striatum of R6/2 mice (Zacharoff *et al*, 2012). In addition, we measured the concentration of other key energy metabolites, such as ATP and inorganic phosphate (P_i), in both Q111 and R6/2 mice using ^{31}P MRS. Finally, we used these measured data to calculate important energy metabolism parameters: the concentration of ADP, phosphorylation potential (PP), the relative rate of ATP synthetase, and the relative rate of creatine kinase (CK) (Nioka *et al*, 1987; Veech *et al*, 1979).

Materials and methods

Animals

Animal breeding, housing, and experiments were performed according to procedures approved by the University of Minnesota Institutional Animal Care and Use Committee. Q111 mutant knock-in huntingtin mice developed and supplied by Dr Marcy MacDonald (Wheeler *et al*, 1999) were bred in heterozygotic crosses. These mice had been previously backcrossed >20 generations onto CD1 mice (Dr Marcy MacDonald, personal communication). Heterozygotic F1 offspring (Q111) and WT controls were scanned at 6 and 13 weeks of age. Timed pregnant female C57B6/CBA mice previously mated with R6/2 males were obtained from the CHDI colony at Jackson Laboratories

(Barr Harbor, ME, USA) and the offspring were scanned at 11 weeks of age.

All mice were housed in mixed genotype groups, 2 to 4 per cage, in enriched conditions. Mice received daily fresh food mash in addition to standard pellet food, Purina 5001 (Menalled *et al*, 2009). Genotyping was performed by Laragen (Los Angeles, CA, USA). CAG repeat numbers ranged from 104 to 112 for Q111 mice and 120 to 142 for R6/2 mice.

^1H and ^{31}P Magnetic Resonance Spectroscopy

Spontaneously breathing mice were fixed in a cylindrical chamber, anesthetized using a flowing gas mixture ($\text{O}_2:\text{N}_2\text{O}=1:1$) containing 1.2% to 1.6% of isoflurane and maintained at 37°C by warm water circulation. The respiratory rate was continuously monitored (SA Instruments, Inc., Stony Brook, NY, USA) during magnetic resonance (MR) scanning and the isoflurane level was adjusted to keep the respiratory rate in the range 80 to 120 per minute to assure stable physiology for data collection. No differences were detected in the respiratory rates or the applied anesthetic doses between HD mice and their respective controls ($P>0.05$) for all conditions. For example, during the ^1H MRS scans of 6-week-old mice, average respiration rates were $103 \pm 23 \text{ min}^{-1}$ (Q111) and $97 \pm 11 \text{ min}^{-1}$ (WT) using isoflurane levels of $1.55 \pm 0.12\%$ (Q111) and $1.50 \pm 0.04\%$ (WT). Comparably equal respiration rates and anesthetic levels were maintained for other ages and genotypes. Typical durations of the studies for a single animal were 1 hour for ^1H MRS and up to 2 hours, when ^{31}P MRS was included. The ^1H MRS data were collected from the striatum of Q111 and WT mice at 6 and 13 weeks ($n=7$ to 9 in each group). The ^{31}P MRS data were collected from the dorsal brain of Q111 and WT mice at 6 and 13 weeks ($n=5$ in each group). R6/2 mice and littermate controls ($n=4$ each) were scanned at 11 weeks with ^{31}P MRS. The ^1H MRS was previously reported from the cortex and striatum of R6/2 mice (Zacharoff *et al*, 2012).

All experiments were performed using a 9.4-T/31-cm horizontal magnet (Varian/Magnex Scientific, Yarnton, UK) equipped with a 15-cm gradient coil (450 mT/m, 200 μs) and a set of strong second-order shims (Resonance Research, Inc., Billerica, MA, USA). The magnet was interfaced to a Varian INOVA console (Varian, Inc., Palo Alto, CA, USA). Proton data were acquired with a transmit/receive quadrature surface radio frequency (RF) coil (14 mm loop diameter). Phosphorus data were acquired with a linearly polarized two-turn 162 MHz ^{31}P coil of 12 mm diameter combined with a 400-MHz quadrature proton coil. A short capillary (0.5 mm inner diameter) filled with water was attached to the ^{31}P coil (perpendicular to the magnet axis) to visualize its location, which was used for precise positioning of the coil relative to the mouse brain.

All first- and second-order shim terms were automatically adjusted using FASTMAP with EPI readout. The ^1H MRS data were acquired from 6.8 to 8.5 μL volumes of interest (VOIs) centered in left striatum at the level of the anterior commissure using an ultra-short echo-time STEAM sequence (repetition time (TR)=5 seconds, echo

time = 2 ms) combined with outer volume suppression and VAPOR water suppression (Zacharoff *et al*, 2012). The striatal water signal linewidths were 10 to 12 Hz. The VOIs were selected based on multi-slice fast spin-echo magnetic resonance imaging in sagittal and coronal orientations. Placement of VOI was not affected by genotype as there is no detectable striatal shrinkage in Q111 mice at this age (Wheeler *et al*, 2000).

The ^{31}P MRS data were acquired from a 75- μL VOI positioned in dorsal brain (including dorsal cortex, hippocampus, striatum, thalamus, and superior colliculus) using an ISIS localization sequence (TR = 5.3 seconds) with broadband adiabatic hyperbolic secant pulses (bandwidth of 8 to 16 kHz). Selection of a substantially larger VOI for ^{31}P MRS relative to ^1H MRS was necessary due to the lower sensitivity of ^{31}P relative to ^1H . In addition, a progressive saturation method (TR = 2 to 10 seconds) was used to estimate T_1 relaxation times of ^{31}P -containing compounds.

Metabolite Quantification

The concentrations of brain metabolites were quantified from ^1H MR spectra using LCModel with the spectrum of fast relaxing macromolecules (MM) included in the basis set (Tkac *et al*, 2007; Zacharoff *et al*, 2012). The MM spectrum was measured from a WT control mouse using the metabolite nulling inversion-recovery technique (TR = 2 s, TI = 0.68 s). The residual signal of the methylene group of phosphocreatine (PCr) with a short T_1 was removed from the free induction decay using Hankel singular value decomposition and the high-frequency noise was suppressed using a Gaussian filter (σ = 0.05 seconds) before including the MM spectrum into the LCModel basis set. The following 17 metabolites were consistently quantified: alanine (Ala), ascorbate (Asc), creatine (Cr), γ -aminobutyric acid, glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycerophosphocholine (GPC), *myo*-inositol (*myo*-Ins), lactate (Lac), *N*-acetylaspartate, *N*-acetylaspartylglutamate, PCr, phosphocholine, phosphoethanolamine (PE), and taurine (Tau). Most metabolites were quantified with Cramér-Rao lower bounds of 4% to 25%, corresponding to estimated errors in metabolite concentrations of 0.2 to 0.6 $\mu\text{mol/g}$. Ascorbate and *N*-acetylaspartylglutamate were quantified with Cramér-Rao lower bounds of <35%. Detection sensitivity was not high enough to reliably resolve GPC from phosphocholine, therefore their sum is reported. The unsuppressed water signal measured from the same VOI was used as an internal reference for the quantification. Brain water content was measured as a difference between the initial brain wet weight and the dry weight after desiccation at 50°C for 7 days. Since no significant difference in brain water content between Q111 and WT mice was found (WT 76.9 $\pm$ 2.3%, n = 6; Q111 75.4 $\pm$ 0.6%, n = 3; P > 0.05), an average value of 76.4% was used for metabolite quantification.

Phosphorus-containing compounds were quantified from Gaussian filtered ^{31}P MRS using the built-in deconvolution software of the Varian console. Ten resonances were assigned to β -ATP, NADP, α -ATP, γ -ATP, PCr, GPC, glycerophosphoethanolamine, P_i , phosphocholine, and PE.

In the R6/2 spectra, the peaks for intracellular and extracellular P_i were also distinguishable from each other. The PCr signal linewidth was 11.2 $\pm$ 1.4 Hz across all scans. Signal intensities were corrected for T_1 saturation effects, using the following T_1 values of ^{31}P metabolites measured by the progressive saturation method: PE, 5.0 seconds; phosphocholine, 5.3 seconds; P_i , 3.0 seconds; glycerophosphoethanolamine, 2.7 seconds; GPC, 3.5 seconds; PCr, 2.7 seconds; γ -ATP, 2.0 seconds; α -ATP, 0.7 seconds; NADP, 0.8 seconds; β -ATP, 1.7 seconds. Given the highly reproducible positions of the RF coil relative to the measured VOI and the identical power calibration results across mice, the RF coil sensitivity was assumed to be the same between individual mice.

^{31}P signal intensities were converted into actual ^{31}P concentrations using a scaling factor calculated assuming a mean PCr concentration of 4 $\mu\text{mol/g}$ in the corresponding control group based on ^1H MRS data. In addition, all ^{31}P concentrations were corrected for the presence of cerebrospinal fluid in the dorsal brain volume sampled.

Reported ATP concentrations were calculated as an average of values determined from α -ATP and γ -ATP resonances. Intracellular pH (pH_i) was calculated by using the chemical shift difference between the P_i and PCr signals (δ_{pi}) and the Henderson-Hasselbalch equation

$$\text{pH}_i = 6.75 + \log(\delta_{\text{pi}} - 3.29)/(5.68 - \delta_{\text{pi}}) \quad (1)$$

(Petroff *et al*, 1985) where δ_{pi} is the chemical shift difference in p.p.m. between P_i and PCr signals. The concentration of Mg^{2+} was assessed from the chemical shift difference between β -ATP and PCr signals ($\delta_{\beta\text{ATP}}$) using the formula:

$$\text{pMg}^{2+} = 4.24 - \log[(\delta_{\beta\text{ATP}} + 18.58)^{0.42}/(-15.74 - \delta_{\beta\text{ATP}})^{0.84}] \quad (2)$$

(Iotti *et al*, 1996).

Calculations of Energy Balance Parameters

The CK reaction was used to assess ADP concentration:

$$[\text{ADP}] = ([\text{Cr}] \times [\text{ATP}]) / (K_{\text{ck}} \times [\text{PCr}] \times [\text{H}^+])$$

(Veech *et al*, 1979)

The CK equilibrium constant, $K_{\text{ck}} = 1.66 \times 10^9$ per mol/L, represents the *in-vitro* value determined from rat brain (Veech *et al*, 1979). Since neither the pH_i nor Mg^{2+} changed in any of the mice (Table 1), the value of K_{ck} was assumed to apply to all genotypes.

The PP was calculated according to

$$\text{PP} = [\text{ATP}] / [\text{ADP}] \times [\text{P}_i] \quad (4)$$

(Veech *et al*, 1979)

The PP represents the immediately available high energy phosphate pool in the brain as determined by mass action.

The relative steady-state rate of ATP synthesis was calculated from:

$$v/V_{\text{max-ATP}} = 1/(1 + 0.2/\text{ADP} + 0.13/[\text{P}_i]) \quad (5)$$

(Nioka *et al*, 1987; Veech *et al*, 1979)

Table 1 Calculated values of intracellular pH and global Mg^{2+} concentration from ^{31}P MRS

Genotype	Age (weeks)	pH_i			$[Mg^{2+}]$ (mmol/L)		
		Mean	s.d.	P	Mean	s.d.	P
Q111	6	7.049	0.039	0.700	0.161	0.015	0.553
WT	6	7.058	0.033		0.169	0.023	
Q111	13	7.048	0.030	0.067	0.143	0.025	0.092
WT	13	7.010	0.025		0.175	0.028	
R6/2	11	6.981	0.019	0.359	0.162	0.014	0.547
WT	11	6.997	0.026		0.167	0.010	

MRS, magnetic resonance spectroscopy; WT, wild type; pH_i , intracellular pH; HD, Huntington disease.

P values from two-tailed *t*-test comparing HD mice with controls.

The relative rate $v/V_{\max-CK}$ of the CK reaction was calculated from:

$$v/V_{\max-CK} = \frac{[ADP]}{([ADP] + K_{m-ADP})} \times \frac{[PCr]}{([PCr] + K_{m-PCr})} \quad (6)$$

(Veech *et al*, 1979)

with $K_{m-ADP} = 0.8$ mmol/L and $K_{m-PCr} = 5.0$ mmol/L (Kuby *et al*, 1954).

All of these energy balance parameters were calculated using mean metabolite concentrations assessed from 1H or ^{31}P MRS data. Concentrations of ATP, PCr, P_i , and pH values were obtained from ^{31}P MRS measurements and assumed to apply to all subregions within the large dorsal brain volume. The estimated ADP concentrations were considered to be regionally selective, because the brain region-specific $[PCr]/[Cr]$ ratios, quantified from the striatal or cortical 1H MR spectra, were used in equation (3). Consequently, the derived energy balance parameters (PP, $v/V_{\max-ATP}$, and $v/V_{\max-CK}$) were as well region specific. The 1H MRS measurements for R6/2 in striatum and cortex were taken from our previous study (Zacharoff *et al*, 2012). The standard deviations of the derived variables were calculated according to propagation of errors formulas using standard deviations for each measured variable (Meyer 1975). Calculated values of [ADP], PP, and relative reaction rates must be understood to be best estimates, since these calculations required combining 1H and ^{31}P MRS data that were acquired from overlapping but not exactly identical brain regions. Nonetheless, since these parameters were calculated using regionally selective striatal or cortical 1H MRS data for the $[PCr]/[Cr]$ ratio, they will be referred to as 'striatal' or 'cortical' values throughout this paper.

Statistical Analysis

For 1H MRS, two different statistical procedures were applied. Only comparisons identified as significantly different in both tests were reported. Initially, two-tailed *t*-tests were used to compare mean metabolite concentrations from striata of Q111 and WT mice of the same age and to compare mean metabolite concentrations of the same strains measured at 6 and 13 weeks. To correct for multiple comparisons, *P* value thresholds $P < 0.01$, $P < 0.002$, and

$P < 0.0002$ were used for the determination of statistical significance. Additionally, *P* values for changes in metabolite concentrations were ranked and evaluated using a false discovery rate procedure. The false discovery rate was set to detect a false positive in only one of the fifty identified metabolite changes ($q = 0.02$). Partial Least Squares-Discriminant Analysis (PLS-DA) was performed using the *pls* package in R (Zacharoff *et al*, 2012). Both mouse strain and age were used as dependent variables, with the concentrations of 17 metabolites as independent variables.

For ^{31}P MRS, *t*-tests were used to compare mean metabolite concentrations between each group of HD mice and its respective control group. For calculated parameters, two-way analysis of variance (ANOVA) was used (Prism, v. 4.0; Graphpad, La Jolla, CA, USA). Power calculations were made using Statmate 2.0 (Graphpad).

Results

1H Magnetic Resonance Spectroscopy in Q111 Mice

At 6 weeks of age, changes in concentrations of some striatal metabolites were already detectable in Q111 mice relative to controls (Figures 1 and 2). Most notably, increased Cr and decreased PCr concentrations (Figure 2A) resulted in a decreased $[PCr]/[Cr]$ ratio (Figure 2C). No change was detected in the total creatine (Cr + PCr) concentration (Figure 2B). A decrease in Tau and a small increase in Glc concentrations were observed. In addition, an increase in the macromolecular content was detected in Q111 mice relative to WT controls (Figure 2A).

When the Q111 mice were scanned again at 13 weeks, the pattern of metabolite changes compared with controls had again changed appreciably. Concentrations of Cr and PCr, as well as Glc and MM, had returned to within normal range (Figure 2A). Only Tau levels remained depressed. Changes not observed at the earlier age included an increase in Gln and a decrease in γ -aminobutyric acid concentrations. In addition to the restoration of [Cr] and [PCr], significant decreases in [PE] and [Glc] were observed at 13 weeks versus 6 weeks.

Considering the large number of metabolites and the colinearity likely to exist among them, PLS-DA analysis was used to determine if the overall profiles of Q111 striatal metabolite concentrations were distinct at each age and compared with the controls (Figure 3A). Principal components were constructed mathematically by combining weighted individual metabolite concentrations to explain variability between groups. Together, two principal components, PC1 and PC2, were needed to distinguish striatal metabolite profiles of 6- and 13-week-old Q111 mice from each other and from controls. The weights or loading values characterized each principal component (Figure 3B). PC1 was largely influenced by the concentrations of Lac, Tau, PCr, Gln, and Cr, while Lac, Gln, Tau, Glc, and PE were major contributors to PC2. PC1 generally distinguished

Figure 1 *In-vivo* ^1H MR spectra acquired from the striatum of Q111 and wild-type (WT) mice at 13 weeks. STEAM, echo time = 2 ms, repetition time (TR) = 5 seconds, VOI = $8.5\ \mu\text{L}$, number of transients = 240. No water signal removal or baseline corrections were applied. Insets: coronal and sagittal FSE MRI with a typical VOI selection. MR, magnetic resonance; VOI, volumes of interest; FSE MRI, fast spin-echo magnetic resonance imaging; Ala, alanine; Asc, ascorbate; Cr, creatine; GABA, γ -aminobutyric acid; Glu, glutamate; Gln, glutamine; GSH, glutathione; GPC, glycerophosphocholine; Ins, inositol; Lac, lactate; NAA, *N*-acetylaspartate; PCr, phosphocholine; Tau, taurine.

between Q111 and controls. PC2 largely separated the two ages of Q111. Metabolite profiles represented in PC1 versus PC1 space overlapped for the WT mice at 6 and 13 weeks but were largely separated for the Q111 mice at these ages (Figure 3A). The ability of PLS-DA to separate genotypes and ages from these very closely related metabolite profiles shows the potential usefulness of metabolite profiles for monitoring disease progression.

^{31}P Magnetic Resonance Spectroscopy in Q111 and R6/2 Mice

Figure 4 shows the excellent quality of ^{31}P MR spectra acquired from Q111 and R6/2 mice and their controls. No changes were detected in the concentrations of ^{31}P compounds between Q111 and control mice at 6 or 13 weeks (Figure 5A). A global decrease in dorsal brain [PCr] was not detected by ^{31}P MRS, suggesting that the decrease in [PCr] observed by ^1H MRS may be regionally specific to the striatum.

In manifest disease in R6/2 at 11 weeks, the majority of ^{31}P -metabolite concentrations were also not significantly different from littermate controls at the $P < 0.05$ level (Figure 5B). Only the concentration of GPC increased in R6/2 dorsal brain ($P = 0.032$)

consistent with previous ^1H MRS measurements (Tkac *et al*, 2007). Both [PCr] and [NADP] tended to increase compared with controls ($P = 0.06$ and $P = 0.053$, respectively). In this large dorsal brain volume, the upward trend in [PCr] was consistent with the small cortical and large striatal increase in [PCr] observed by ^1H MRS of R6/2 mice (Zacharoff *et al*, 2012).

In addition to quantification of the concentrations of phosphorus-containing compounds, pH_i was calculated from the chemical shift differences between resonances of P_i and PCr. The pH_i of Q111 mice was not statistically different from that of the WT mice and did not exhibit any age-dependent changes (Table 1). The values of pH_i also did not differ between the R6/2 brains and those of littermates. Similarly, the Mg^{2+} levels were calculated from the chemical shift differences between the resonances of PCr and β -ATP. The concentration of Mg^{2+} did not differ between Q111 and WT mice or between R6/2 and WT mice (Table 1), consistent with the absence of change in [ATP].

Calculated Parameters for Energy Metabolism

The calculated concentration of ADP in the striatum of Q111 mice was increased by 79% compared with

Figure 2 Striatal neurochemical profiles determined from ^1H magnetic resonance spectroscopy (MRS) of Q111 and wild-type (WT) mice at 6 and 13 weeks of age. (**A**, **B**) metabolite concentrations, (**C**) concentration ratio, data represent mean $\pm$ s.d., $n = 7$ to 9. Significance level for the comparison Q111 versus WT of the same age: $*P < 0.01$, $**P < 0.002$, $***P < 0.0002$. Significance level for the age comparison for each genotype: $^{\alpha}P < 0.01$; $^{\beta}P < 0.002$; $^{\gamma}P < 0.0002$. (**C**) For [PCr]/[Cr], two-way analysis of variance (ANOVA) attributed 37.9% of the variance to genotype, $P < 0.0001$, 6.9% to age, $P < 0.045$ and 8.1% to their interaction, $P < 0.031$. Bonferroni posttest comparisons. Ala, alanine; Asc, ascorbate; Cr, creatine; GABA, γ -aminobutyric acid; Glc, glucose; Glu, glutamate; Gln, glutamine; GSH, glutathione; GPC, glycerophosphocholine; myo-Ins, myo-inositol; Lac, lactate; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; PE, phosphoethanolamine; PCr, phosphocholine; Tau, taurine; MM, macromolecules.

Figure 3 Partial Least Squares-Discriminant Analysis (PLS-DA) analysis of metabolite concentrations measured by ^1H Magnetic Resonance Spectroscopy (MRS) from striata of Q111 and wild-type (WT) mice at 6 and 13 weeks. (**A**) The score plot of PC1 versus PC2 discriminated Q111 metabolite profiles at both ages from those of the WT mice. PLS-DA data of 6 and 13 weeks WT mice overlapped. The proportion of the variability explained by each component is indicated in parentheses on each axis. (**B**) The loading plots show the weightings or relative contributions of each metabolite in constructing the principal components. Ala, alanine; Asc, ascorbate; Cr, creatine; GABA, γ -aminobutyric acid; Glc, glucose; Glu, glutamate; Gln, glutamine; GSH, glutathione; GPC, glycerophosphocholine; myo-Ins, myo-inositol; Lac, lactate; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; PE, phosphoethanolamine; PCr, phosphocholine; Tau, taurine; MM, macromolecules.

Figure 4 Representative *in-vivo* ^{31}P MR spectra measured from (A) Q111 and wild-type (WT) brains at 13 weeks and (B) R6/2 and WT brains at 11 weeks. ISIS localization technique, repetition time (TR) = 5.3 seconds, number of transients = 640. Insets: coronal and sagittal FSE MRI with a typical selection of the VOI = $5 \times 3 \times 5 \text{ mm}^3$. MR, magnetic resonance; FSE MRI, fast spin-echo magnetic resonance imaging; VOI, volume of interest; PC, phosphocholine; GPC, glycerophosphocholine; PE, phosphoethanolamine; PCr, phosphocholine; P_i , inorganic phosphate.

Figure 5 Concentrations of phosphorus-containing compounds quantified from brains of Q111 and wild-type (WT) brains at 6 and 13 weeks (A) and R6/2 and WT brains at 11 weeks (B). Bars represent mean $\pm$ s.d.; $n = 4$ for R6/2; $n = 5$ for Q111. Significance level: * $P < 0.05$. GPC, glycerophosphocholine; PE, phosphoethanolamine; PCr, phosphocholine; P_i , inorganic phosphate; PC, phosphocholine; GPE, glycerophosphoethanolamine.

control at 6 weeks and returned to within the range of control values by 13 weeks (Figure 6A). The concentration of ADP in Q111 mice at 13 weeks was

significantly lower relative to 6 weeks. Consistent with this, the PP, a measure of the potential for meeting additional demand, was decreased in Q111

Figure 6 Calculated energetic parameters for Q111, R6/2 and corresponding wild-type (WT) mice for the indicated regions and ages. Data for each Q111 and R6/2 cohort were compared in separate two-way analyses of variance (ANOVAs) with Bonferroni posttests. Significance level comparing transgenic mice with their wild-type counterparts (open bars to left): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; comparisons across age indicated by horizontal lines: $\wedge P < 0.05$, $\wedge\wedge P < 0.01$. (A) ADP concentration. Q111, two-way ANOVA: genotype accounts for 40.2% of variance, $P = 0.0007$, age 14.7%, $P = 0.022$. (B) Relative rates of oxidative phosphorylation. Q111, two-way ANOVA: genotype accounts for 45.6% of variance, $P = 0.0003$, age 13.5%, $P = 0.025$. (C) Phosphorylation potential. Q111, two-way ANOVA: genotype accounts for 41.6% of variance, $P = 0.0007$, age 20.8%, $P = 0.009$. (D) Relative rates of the creatine kinase (CK) reaction. Q111, two-way ANOVA: genotype accounts for 26.5% of variance, $P = 0.015$. R6/2, two-way ANOVA: genotype accounts for 41.9% of variance, $P = 0.006$.

striatum compared with control (Figure 6C). In contrast, no changes in ADP concentration or PP were detected in R6/2 mice at 11 weeks compared with control, either in striatum or in cortex. Both the relative rate of ATP production, $v/V_{\max-ATP}$, and the relative rate of CK, $v/V_{\max-CK}$, were increased in Q111 at 6 weeks compared with control (Figures 6B and 6D) and returned to normal at 13 weeks. No change was observed in R6/2, except for a small increase in $v/V_{\max-CK}$ in cortex.

Discussion

To the best of our knowledge, this is the first study of brain metabolism in Q111 mice using MRS, and also the first measurements in the brain of HD mouse models using ^{31}P MRS. We observed significant changes in [PCr]/[Cr] ratio in the brain of Q111 mice at a young age (6 weeks), suggesting impairment in brain energy metabolism very early in this slowly progressive mouse model. These changes would have occurred before massive nuclear accumulation (2.5 months), aggregate formation (5 months), cellular shrinkage, or behavioral deficits (Menalled *et al*, 2009; Wheeler *et al*, 2000, 2002).

Surprisingly, these changes were largely reversed at 13 weeks. Similarly, both calculated [ADP] and $v/V_{\max-ATP}$ were increased in Q111 at 6 weeks compared with control, and returned close to control values at 13 weeks. In contrast, in symptomatic R6/2 mice at 11 weeks, calculated brain energy parameters were similar to control. This suggests that the homeostatic regulation in brain energy metabolism observed at an early stage in Q111 mice may be maintained through manifest disease.

Early Changes in Metabolite Concentrations in Q111 Presage Disease

The changes in striatal [PCr]/[Cr] ratio in Q111 mice at 6 weeks were largely reversed at 13 weeks and were not detected in R6/2 (Zacharoff *et al*, 2012), suggesting that early changes are followed by homeostatic regulation that return the [PCr]/[Cr] ratio to the normal range. Such early metabolic adjustments would have been missed in previous mouse studies during manifest disease (Jenkins *et al*, 2005). The decrease and subsequent recovery in PCr concentration observed in Q111 mice contrasts with the increase in PCr concentration observed in R6/2

mice (Zacharoff *et al*, 2012), suggesting that multiple disease-related energetic changes may occur with different pathogenic time courses. Indeed, an increase in cortical but not in striatal PCr levels has been reported in Q111 at 4 months, an older age than used here (Mochel *et al*, 2012a). Thus, the processes producing increases in total creatine in manifest disease in R6/2 may just be beginning at 4 months in the Q111 model.

In addition to Cr and PCr, significant changes were also detected in Gln and Tau. The early and progressive increase in striatal [Gln] in Q111 mimicked similar changes reported in R6/2 (Tkac *et al*, 2007; Zacharoff *et al*, 2012). The decrease in [Tau] in striatum of Q111 mice contrasted markedly with its substantial increase in R6/2 (Tkac *et al*, 2007; Zacharoff *et al*, 2012). The promoter region of the Tau transporter contains consensus sites for Sp1 and p53 binding, which reciprocally regulate its expression (Han *et al*, 2006). Sp1 inhibition (Dunah *et al*, 2002) and p53 activation by muhtt (Steffan *et al*, 2000) could account for down regulation of Tau and its transporter at early ages. Finally, the small, but statistically significant increase in the macromolecular content observed at 6 weeks in striatum of Q111 mice could indicate an increased contribution from the soluble, i.e., MRS detectable, muhtt protein.

Previous ^1H MRS investigations of HD mice have all reported decreases in *N*-acetylaspartate levels (Jenkins *et al*, 2005; Zacharoff *et al*, 2012). Therefore, the absence of changes in [*N*-acetylaspartate] observed in this study confirmed that disease progression in Q111 mouse model of HD had not yet produced substantial pathology at the ages studied. Our ability to detect metabolite changes at such early ages and our ability to use this information in PLS-DA to discriminate transgenic animals from controls support the possibility of using ^1H MRS metabolite profiles as biomarkers of disease progression. Note that, for some metabolites, a change in concentration may reflect systemic processes rather than altered brain metabolism or neurochemistry. For example, it is well known that brain glucose concentration depends on plasma concentration (Duarte and Gruetter 2012). Therefore, principal components should be viewed as a composite index that may reflect a combination of brain and systemic processes.

Longitudinal studies on HD mice extending from presymptomatic ages to manifest disease will be necessary to determine principal component sets that could distinguish transgenic animals from littermates and accurately track disease progression. Alternatively, a single metabolite such as Gln, which appears to change in both slowly developing and rapid mouse models, could be used as a biomarker.

Our results in HD mouse models raise the possibility that similar MRS biomarkers could be identified in humans. In addition, as shown here in Q111 mice, studies in HD carriers at an earlier age (childhood or early adulthood) may help uncover biochemical changes that are no longer detectable later during

the premanifest period. So far, clinical ^1H MRS studies in humans have shown changes in *N*-acetylaspartate, *myo*-Ins, and total creatine, but such changes were observed only in manifest disease (Sturrock *et al*, 2010; van den Bogaard *et al*, 2011). Proton MRS at 4 and 7 T has the potential to quantify 12 to 17 metabolites (Emir *et al*, 2011; Tkac *et al*, 2009). However, even recent ^1H MRS studies in HD patients have reported concentrations of only 5 to 6 metabolites at 3 and 7 T, respectively (Sturrock *et al*, 2010; van den Bogaard *et al*, 2011). Therefore, further improvements in clinical MRS protocols may be necessary to realize the full potential of ^1H MRS in HD patients.

ATP Homeostasis

The absence of any detectable changes in ATP concentrations in either Q111 or R6/2 attests to the strong regulation governing this important energetic molecule. Our data are consistent with a study showing that muhtt associates with degenerating nerve terminal mitochondria and decreases synaptosomal, but not whole brain, ATP concentrations in Q140 mouse brain at 14 months (Orr *et al*, 2008). In addition, no difference in ATP concentrations was observed in the occipital cortex of early stage HD patients compared with healthy controls using *in-vivo* ^{31}P MRS (Mochel *et al*, 2012b). However, due to the limited sensitivity of ^{31}P MRS, the present study did not have the power to detect changes in ATP smaller than 10%. Power calculations using the standard deviation of the ^{31}P MRS ATP measurements from R6/2 brains indicated that our study had an 80% ability to detect a 12% ATP decline at the $P=0.05$ level. At the earlier predisease stage in the Q111 mice where variability was increased, 20 or more mice would have been needed to see a 10% change in ATP with comparable confidence.

A recent study (Mochel *et al*, 2012a,b) reported a 6% to 11% decrease in ATP levels measured with high-performance liquid chromatography in the cortex and striatum of diseased R6/2 mice using 14 to 18 animals per group. In contrast, the authors reported no change in ATP concentration in Q111 mice, consistent with our study. The apparent discrepancy between the two studies in R6/2 mice could be explained in a number of ways. First, our study did not have the power to detect a small decrease in ATP. Second, due to the relatively low sensitivity of *in-vivo* ^{31}P MRS, we measured ATP concentrations in a larger volume and we cannot exclude small changes in more localized regions. The main advantage of *in-vivo* MRS is that it permits noninvasive measurements in intact animals.

The absence of changes in ATP did not rule out metabolic compromise in the HD mice. In brain extracts from 20-week-old N171-82Q mice, both glycolytic flux and ATP concentration exceed those from control mice despite decreases in GAPDH

activity (Olah *et al*, 2008). In positron emission tomography studies of presymptomatic and early stage patients, the ratio of oxygen consumption to glucose utilization was elevated to the theoretical limit due to a decrease in glucose utilization, consistent with a defect in glycolysis but not oxidative phosphorylation (Powers *et al*, 2007). Thus even with compromised elements, the energy generating system continues to operate in a regulated manner. Although the basal energetic status throughout disease progression may be challenged and perturbed by muhtt, in both R6/2 and Q111 models, it was largely maintained by intact homeostatic responses that preserved a constant level of ATP.

Relative Rate of ATP Synthesis ($v/V_{\max}(\text{ATP})$)

Calculated $v/V_{\max}(\text{ATP})$ was increased in Q111 mice at 6 weeks, and returned to normal at 13 weeks. This suggests that mitochondria ‘worked harder’, closer to maximal capacity at 6 weeks. Our study did not allow us to determine if this is due to increased consumption (v) or decreased production capacity ($V_{\max}$). Although $V_{\max}(\text{ATP})$, a value indicative of the maximal rates of oxidative phosphorylation, could not be calculated from the current ^1H and ^{31}P MRS measurements, the 13% to 26% increase in the relative rate of ATP generation in Q111 mice was consistent with a trend toward increased mitochondrial respiration from cultured Q150 striatum (Oliveira *et al*, 2007). Using the 69% reduction in maximum respiratory rate ($V_{\max}$) of StHdh111/111 compared with StHdh7/7 cells (Milakovic and Johnson 2005), we estimated the rate of ATP generation (v) in the brain of Q111 mice to be between 79% and 89% of controls at 6 or 13 weeks, respectively. If this applies *in vivo*, then actual ATP generation (v) could be lower than control even though the relative velocity $v/V_{\max}$ is increased. Determining $V_{\max}(\text{ATP})$ *in vivo* will require further investigation.

Similarly, the apparent normalization of $v/V_{\max}$ at 13 weeks in Q111 brains or its apparent lack of change in R6/2 may not mean that both v and $V_{\max}$ are normal. If both v and $V_{\max}$ change in the same proportion, then the relative rate $v/V_{\max}$ would be identical to controls. To determine if the absolute fluxes are different, the magnetization transfer technique could be used in future studies. Finally, even though ^{31}P concentrations were measured in a larger volume, we verified that small regional variations in ^{31}P concentrations would not significantly change the calculated parameters and the conclusions of the present paper.

Changes in the Creatine Kinase Reaction

As with $v/V_{\max}(\text{ATP})$, calculated $v/V_{\max}(\text{CK})$ was increased in Q111 brain at 6 weeks, and returned to normal at 13 weeks. In contrast, it was not increased in the striatum of R6/2 mice, and was only slightly

increased in the cortex of R6/2 mice. Such an increase in $v/V_{\max}(\text{CK})$ might be attributable to downregulation of CK and GAMT expression (Kim *et al*, 2010; Mochel *et al*, 2012a). One difference between the Q111 and R6/2 mouse models is that total creatine (Cr+PCr) was constant in Q111 mice, but increased markedly over time in R6/2 (Zacharoff *et al*, 2012). This increase in total creatine is remarkable considering the reported decrease in enzyme expression. The maximum velocity of the CK reaction would be expected to decline in proportion to the decreased expression, further exacerbating energy storage and retrieval. The cause of the increased total creatine is unknown, and could be a consequence of increased ATP production, altered creatine kinase kinetics, loss of CK or some other cellular process. To date, protein expression levels of CK and its metabolic and catabolic enzymes have not been examined in Q111. Another possible explanation is that, with less CK present, PCr may be trapped in the storage pool, unable to supply ATP in response to demand. Alternatively, the increased total Cr may be attributable to its accumulation as one of many osmolytes that increase in R6/2 brain (Tkac *et al*, 2007; Tsang *et al*, 2006). Natural osmolytes, as well as increased energy availability, reduce muhtt aggregation (Borwankar *et al*, 2011; Wang *et al*, 2009).

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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