^{13}C MRS measures the regional changes in neuronal (Glu/GABA) and astroglial mitochondrial TCA cycle and neurotransmitter cycling of the R6/2 Huntington's disease mice

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INTRODUCTION: Alterations in brain energy metabolism, including reduced glucose utilization and mitochondrial respiration, is observed in Huntington's Disease (HD) and HD animal models (1). Despite our detailed knowledge of the mutated gene and its poly-glutaminated (poly-Q) protein product, mutant huntingtin (mHtt), the mechanism underlying pathology and progression of HD remains enigmatic. There is an acute compelling need to apply new experimental tools to broaden the search for disease mechanism and identify potential therapeutic targets. Magnetic Resonance Spectroscopy (MRS) offers one such tool, which has been applied recently in HD afflicted individuals and HD animal models (1,2). MRS can be readily adapted to measure metabolic pathway flux by use of ^{13}C-labeled substrates In this study we measured the flow of ^{13}C label into glutamate, glutamine and GABA in 6 or 8 week old R6/2 and non-carrier control mice following timed intravenous infusions of [1,6-$^{13}C_2$]glucose (neuronal and glial substrate) or [2-^{13}C]acetate (glial substrate) to determine whether dynamic turnover of the major amino acids linked to brain energy metabolism and neurotransmission (glutamate, GABA and glutamine) is altered in the R6/2 mouse model of HD.

METHODS: R6/2 mice (B6CBA-Tg (Hdexon1)62Gpb/3J) and non-carrier litter mate control mice were obtained from Jackson Laboratories, Bar Harbor, ME at 4 weeks and 6 weeks. Mice were allowed to habituate for one week before surgery to place a jugular vein catheter, followed by one week of post-surgical recovery before experiments at 6 or 8 weeks of age, respectively. Mice were infused with either [1,6-$^{13}C_2$]glucose (8 min) or [2-^{13}C]acetate (15 min), rapidly raising the respective concentrations and ^{13}C-enrichments to constant values (3,4). At the appropriate times, mice were quickly sedated (<30s) with isoflurane, loaded into a specialized holder and euthanized by a focused-beam microwave irradiation device, arresting brain metabolism in <1 sec, allowing brain tissue removal from cortex, striatum and thalamus without postmortem effects (5). Brain tissues were extracted using ethanol with [2-^{13}C]glycine added as an internal concentration standard (4). The concentration and ^{13}C enrichment of amino acids in the extract were determined using ^{1}H-[^{13}C] MRS at 11.74T (Bruker AVANCE) (6).

RESULTS: *Cortical amino acid levels in 8 week old R6/2 and control mice.* The total concentrations of eleven amino acids and metabolites were measured in cortex, striatum and thalamus of 8 week old R6/2 and control mice infused with ^{13}C-labeled glucose or acetate. Consistent changes were seen with both substrates in five of the eleven neurochemicals measured in cortex (glutamine, aspartate, NAA, taurine and myo-inositol; $P<0.05$), striatum (glutamine, myo-inositol; $P<0.05$) and thalamus (taurine; $P<0.05$). With the exception of glutamate, which was lower in cortex (-8%) of R6/2 mice at 8 weeks, all other changes involved increased levels (glutamine, 10-20%; myo-inositol, 12-20%; taurine, 10-14%; total creatine, 14%) in the three brain regions.
*Comparisons between ^{13}C labeling in 6 and 8 week old R6/2 mice***.** Figure 1 shows the percent changes relative to non-carrier controls of altered ^{13}C labeling from [1,6-^{13}C2]glucose or [2-^{13}C]acetate measured for cortex, striatum and thalamus of R6/2 mice at 6 and 8 weeks of age. Reduced ^{13}C labeling from glucose was seen in glutamate-C4, GABA-C2 and glutamine-C4 in all three brain regions at 6 weeks, which became more pronounced by 8 weeks. These changes are indicative of a reduction of the neuronal TCA cycle in GABAergic and glutamatergic neurons. In mice infused with acetate, reduced ^{13}C labeling was seen in glutamate-C4, GABA-C2 of striatum and thalamus but not cortex at 6 weeks and all three regions at 8 weeks consistent with a reduction in glutamate and GABA/glutamine cycling. Glutamine-C4 labeling in contrast was relatively unaffected suggesting minimal impact on glial metabolism.

CONCLUSIONS: R6/2 mice showed significant differences both in the levels of several neurochemicals and the extent of ^{13}C incorporation from ^{13}C-labeled glucose and acetate into glutamate, GABA and glutamine of the three brain regions examined. Decreased ^{13}C labeling (and metabolic fluxes) were more pronounced in 8 week old than in 6 week old R6/2 mice, consistent with the know worsening of HD symptoms in this mouse model. In contrast to the reduction in neuronal glucose oxidation seen at 6 weeks, astroglial oxidative metabolism was relatively spared as revealed by no change in glutamine-C4 labeling, with exception of a decline in thalamus at 8 weeks. The reduced labeling of glutamate-C4 and GABA-C2 labeling from [2-^{13}C]acetate indicates reduced rates of glutamate/GABA neurotransmission accompanying the decline in energy metabolism.

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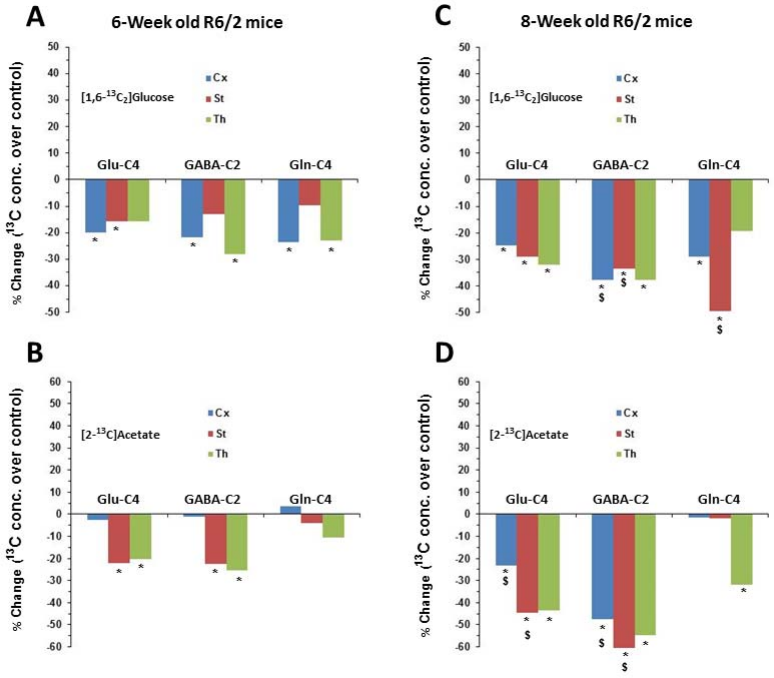

FIGURE 1: Percent changes of ^{13}C concentration (μmol/g) of glutamate-C4, GABA-C2 and glutamine-C4 measured in cell-free extracts of cortex (Cx), striatum (St) and thalamus (Th) of 6 week old or 8 week old R6/2 mice relative to age and litter-matched non-carrier controls following intravenous infusion of either [1,6-$^{13}C_2$]glucose (8 min) or [2-^{13}C]acetate (15 min), respectably. Values represent the mean of 5-6 mice per group (*$P<0.05$ relative to controls at same age, $^{\$}P< 0.05$ between 6 and 8 weeks).