Dietary Restriction Recovers Cerebral Activity in Pitx3 knockout Mouse Model of Parkinson's Disease

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Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Pitx3 knockout (Pitx3$^{-/-}$) mouse, which exhibits malformation of substantia nigra leading to loss of dopamine innervations in striatum, is a well characterized genetic model of chronic PD[1]. Dietary restriction (DR) is linked with longevity and neuroprotection on laboratory animals via reduced oxyradical formation[2] and induction of expression of cytoprotective stress proteins[3]. The objective of the current study is two folds: 1) to understand the effects of loss of dopaminergic neurons on the cerebral metabolism, and 2) to evaluate the potential of DR in improving the function of glutamatergic and GABAergic neurons in different brain regions by using ^{1}H-[^{13}C]-NMR spectroscopy in conjunction with infusion of [1,6-$^{13}C_2$]glucose.

Materials and Methods: Four groups of six month old male mice; Group A: Wild Type Control (n=5); Group B: Wild Type + DR (n=4); Group C: Pitx3$^{-/-}$ Control (n=5) and Group D: Pitx3$^{-/-}$ + DR (n=5) were used for the study. Group B and D mice were subjected to DR by providing food every alternate day for six months (~ 60-70% calorie intake of *ad libitum*), while Group A and C mice had free access to food. For metabolic studies, overnight fasted mice were anesthetized with urethane and infused with [1,6-$^{13}C_2$]glucose (i.v.) for 10 min[4]. At the end of the experiment, head was frozen *in situ* in liquid N_2 and metabolites were extracted from frozen cortical and striatal tissue[5]. ^{1}H-[^{13}C]-NMR spectra were acquired from tissue extracts for the measurement of concentration and ^{13}C enrichment of amino acids[6].

Results and Discussion: GABA level was found to be increased (Control 5.9±1.2; Pitx3$^{-/-}$ 7.7±0.9 μmol/g, p=0.015) in striatum of Pitx3$^{-/-}$ mice (elevated levels of GABA in striatum has been reported in Parkinson's patients), which returned to normal level (Control+DR 6.9±0.7; Pitx3$^{-/-}$+DR 6.2±0.6 μmol/g, p=0.126) following DR intervention. ^{13}C Labeling of Glu_{C4} (Control 1.99±0.21; Pitx3$^{-/-}$ 1.44±0.18 μmol/g, p=0.0007), $GABA_{C2}$ (Control 0.19±0.04; Pitx3$^{-/-}$ 0.11±0.02 μmol/g, p=0.002) and Gln_{C4} (Control 0.23±0.07; Pitx3$^{-/-}$ 0.13±0.03 μmol/g, p=0.017) from [1,6-$^{13}C_2$]glucose was found to be decreased significantly in cerebral cortex of Pitx3$^{-/-}$ mice (Fig. 1A) suggesting reduced glucose oxidation by glutamatergic and GABAergic neurons and neurotransmitter cycling. Hypo-metabolism and reduced neurotransmitter cycle was also observed in the striatal region (Fig. 1B). The reduced glutamatergic and GABAergic glucose oxidation and neurotransmitter cycle in cortical and striatal regions in Pitx3$^{-/-}$ mice could recover to control level following DR (Fig. 1A & B). The normalization of GABA level and energy metabolism with DR in Pitx3$^{-/-}$ mice suggest that DR has potential for improving brain function in abnormal conditions which could be used as a strategy for the management of PD in combination with conventional drug treatment.

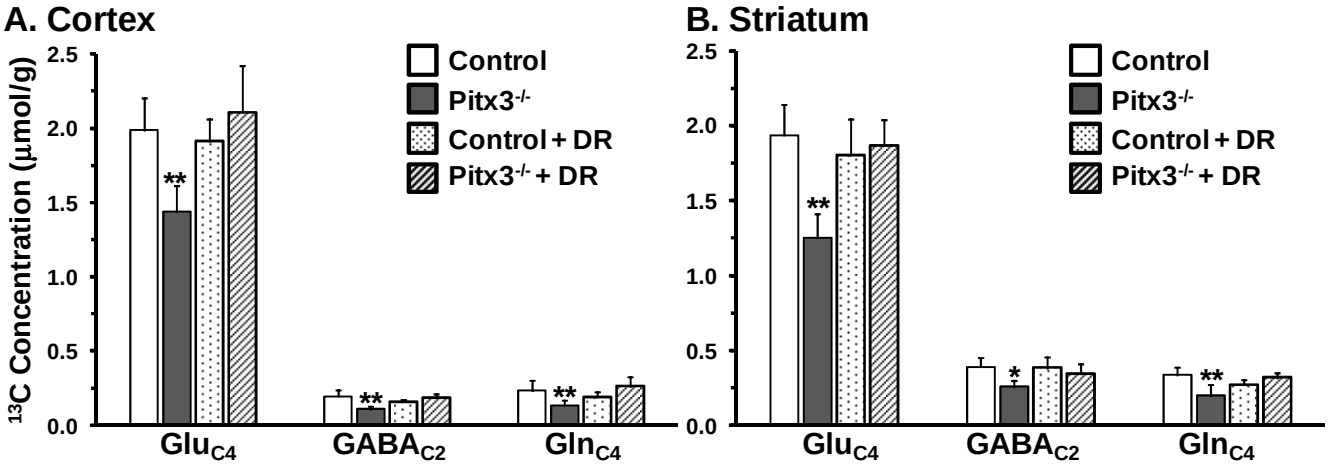

Fig. 1. Concentration of ^{13}C labeled amino acids from [1,6-$^{13}C_2$]glucose at 10 min. *p< 0.01, **p<0.001 when compared with control or Pitx3$^{-/-}$+DR

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