INTRODUCTION: Alzheimer’s disease (AD) is one of the most common forms of neurodegenerative disorders characterized by progressive memory impairment, disordered cognitive functions marked with deficits in numerous neurotransmitters with progression of disease. Glucose, the most important energy source in the brain, is metabolized via the pyruvate dehydrogenase (PDH), pentose phosphate (PPP) and pyruvate carboxylase (PC) pathways. APP-PS1 mouse, a model of AD, exhibit enormous plaque loading and memory impairment at the late age - pathology similar to human AD. Glutamate and GABA, the major excitatory and inhibitory neurotransmitters, account for the major fraction of glucose oxidation in brain\(^2\). Earlier study has shown that flux through PDH pathway is significantly impaired in APP-PS1 mice at the preclinical stage of AD\(^3\). However, the fate of PC and PPP pathways in AD is not explored. The present study evaluates the fluxes through PC and PPP in APP-PS1 mouse brain using \(^{13}\)C NMR spectroscopy together with infusion of \([2-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose.

MATERIALS AND METHODS: APP-PS1 (20 month) and age matched control mice were used for the study. Overnight fasted APP-PS1 and control mice were anesthetized with urethane and infused with \([2-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose for 30 min\(^4\). Metabolism of \([2-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose via pyruvate dehydrogenase (PDH) pathway incorporates label into Glu\(_{C5}\), Gln\(_{C5}\) and GABA\(_{C1}\), while its metabolism via PC pathway incorporates label into Gln\(_{C3}\), Glu\(_{C3}\) and GABA\(_{C3}\). Metabolism of \([2-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose via pentose phosphate pathway labels Glu\(_{C4}\), Gln\(_{C4}\) and GABA\(_{C2}\). In addition, mice were also administered \([1,6-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose for 10 and 90 min to investigate glutamatergic and GABAergic neuronal TCA cycle flux. At the end of the infusion, mice brain was frozen in situ using liq N\(_2\), and metabolites were extracted from frozen brain\(^5\). After preliminary NMR analysis (\(^{1}H-{\text{\textsuperscript{13}}}\text{C}-NMR\) and \(^{1}H-{\text{\textsuperscript{15}}}\text{H}-NMR\) of tissue extracts, glutamate and glutamine were separated by passing the extract through AG 1-X8 anion exchange column\(^6\). \(^{13}\)C labeling of amino acids were measured in separated fractions using \(^{1}H-{\text{\textsuperscript{13}}}\text{C}-NMR\) spectroscopy\(^7\). \(^{13}\)C NMR signal intensity together with measured percent labeling of amino acids at internal carbon positions from \(^{1}H-{\text{\textsuperscript{13}}}\text{C}-NMR\) was used to estimate the percent labeling of amino acids at terminal carbon.

RESULTS AND DISCUSSIONS: The \(^{13}\)C labeling of Glu\(_{C4}\), GABA\(_{C2}\), Gln\(_{C4}\), Asp\(_{C3}\), Gln\(_{C3}\) from \([1,6-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose infusion was found to be significantly (F[1,4]=65, p=6.3e\(^{-10}\)) lower in APP-PS1 mouse than age matched control indicating glucose oxidation associated with glutamatergic and GABAergic neurons is impaired in APP-PS1 mice (Fig. 1). \(^{13}\)C-\(^{1}H\)-NMR spectrum obtained from \([2-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose infusion shows labeling of amino acids at different carbon positions arising from PPP, PC and PDH pathway (Fig. 2). The ratio, \([\text{Gln}_{C3}]+[\text{Glu}_{C3}]\)/\([\text{Glu}_{C5}]\) which depicts PC/PDH was found to be 0.139±0.017 and 0.153±0.024 in APP-PS1 and control mice, respectively. This together with the rate of glucose oxidation obtained from \([1,6-{\text{\textsuperscript{13}}}\text{C}]{\text{\textsuperscript{15}}}\)glucose study in APP-PS1 and control mice indicates reduction of PC flux (APP-PS1 0.030±0.004; Control 0.055±0.009 \(\mu\)mol/g/min, p=0.003) in APP-PS1 mice. PPP flux obtained from the ratio Glu\(_{C4}/\)Glu\(_{C5}\) and glucose oxidation rate was also found to be reduced significantly (P=0.001) in Alzheimer’s mice (APP-PS1 0.010±0.002; Control 0.021±0.003 \(\mu\)mol/g/min). These data indicate that in addition to reduced glutamatergic and GABAergic activity, flux through PC and PPP is also impaired in AD mice at the late age.


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