Caloric Restriction Enhances Oxidative Brain Metabolism in Healthy Aging Detected by $^{1}H[^{13}C]MRS$

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Introduction: Brain function plays a crucial role in lifespan determination (1). Preserving brain function and metabolism with age is critical for optimizing healthspan and lifespan. Caloric restriction (CR) is the most studied anti-aging manipulation and has been shown to increase the lifespan of a broad range of species. Rats (F344BNF1) treated with CR also showed enhanced memory (2). However, the effects of calorie restriction on aging brain metabolism and neuronal activity remain largely unexplored. In the study, we used in vivo proton observed carbon edit (POCE) or $^{1}H[^{13}C]$ MRS to characterize the effect of CR on the rates of neuronal TCA cycle flux ($V_{TCA,N}$) and total glutamatergic neurotransmission from the glutamate and glutamine cycle flux ($V_{cyc(tot)}$) in aged F344BNF1 rats. We hypothesized that CR rats may have enhanced brain bioenergetics with aging.

Material and Methods: Control and CR aged male rats (N= 6 for each group, 24 months old) were purchased from NIA. Animals were anesthetized with alpha chloralose. In vivo $^{1}H[^{13}C]$ NMR study was performed on a Varian 11.7T MR system with POCE sequence. The POCE spectra were obtained from a localized volume (8x4x 6 mm^3) that covered cortex and hippocampus (Fig. 1A). [1,6-^{13}C] labeled glucose was continuously infused via the femoral vein of the rat for 120 min and POCE spectra were acquired simultaneously (Fig. 1B). Six blood samples were taken from a femoral artery during the 2-hour scan to determine the [1,6-^{13}C] labeled glucose level. The concentrations of the metabolites were also determined in the brain extracts at the end point of the labeled isotope infusions. Data were analyzed with the CWave program for mathematical modeling to determine the TCA cycle rate in neurons ($V_{TCA,N}$) and glutamate-glutamine cycle rate ($V_{cyc(tot)}$). T-test was used to determine the difference of the measured indices between the control and CR rats.

Resutls: Figs 1C and D show the POCE turover data (and model fits) of [4-^{13}C] glutamate and [4-^{13}C] glutamine in CR and control rats, respectively. Dots represent measured fractional enrichment as obtained from data, wherease the solid line reprents the best mathematical fit to the metabolic model through CWave. The CR rats showed higher rates compared to the controls. Interestingly, we also found that the ^{13}C fractional enrichment of [1,6-^{13}C] labeled glucose in the blood samples had no significant differences between the two groups. As a result, aged CR rats had significantly higher $V_{TCA,N}$ ($P < 0.0001$) and $V_{cyc(tot)}$ ($P < 0.001$) relative to controls (Fig 1E). Our results demonstrated that $V_{TCA,N}$ and $V_{cyc(tot)}$ has a ~ 2:1 ratio, similar to literaure value for a variety of other conditions examined, from awake to different anesthetized states (3). We also found that aged CR rats had similar $V_{TCA,N}$ (~ 0.4 µmol/g/min) and $V_{cyc(tot)}$ (~ 0.22 µmol/g/min) relative to those of the younger control rats as reported in literature (4).

Discussion: We demonstrated that long-term CR (the rats have been put on CR since 16 weeks old) has significant effects on brain metabolism in cortex and hippocampus. Aged CR rats had enhanced neuronal metabolism and that was similar to young ones, suggesting that CR may delay brain age-related metabolic reduction in rodents. Our observation is also consistent with the findings that CR rats had enhanced memory that involves hippocampus function. Collectively, it suggests that CR can extend healthspan and thus lifespan. CR rats did not have significant %PE of glucose level compared to controls, but had higher metabolic rates, suggesting that CR rat's brain may use alternative fuel substrate, such as ketone bodies, to meet the energy demand (5). As ketone bodies are known to be neuroprotective, the shift in the metabolic pathway (from glucose to ketone bodies) may be another contributing factor for extended healthspan and lifespan of the CR rats. Future studies are needed to explore on this topic. The understanding of the interplay between CR, ketone body metabolic pathway and brain function may have profound implications on retarding aging and age-related neurodegenerative disorders.

References: (1) Mattson et al., Ageing Res Rev 1:155 (2002); (2) Carter et al., J Gerontol A Biol Sci Med Sci. 64A: 850 (2009); (3) Hyder et al., J, Cereb Blood Flow & Metab. 26:865 (2006); (4) Ennis et al., Neurochem Res. 36:1962 (2011); (5) Maalouf et al., Brain Res Rev 293-315 (2009).

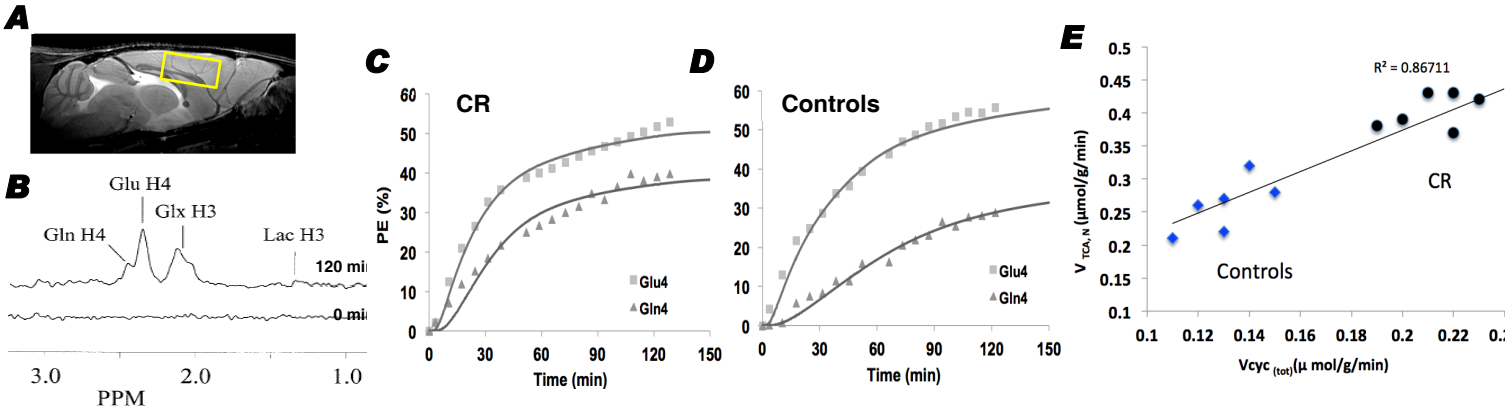

Figure1. (A) The imaging voxel (included cortex and hippocampus) for the POCE experiment. **(B)** Time-resolved $^{1}H[^{13}C]$ spectra following the onset of [1,6-^{13}C]-glucose infusion at 0 (beginning) and 120 min (end point). **(C)** and **(D)** POCE turnover curves of [4-^{13}C] glutamate. and [4-^{13}C] glutamine in CR and control rats. **(E)** Determined $V_{TCA,N}$ and $V_{cyc(tot)}$. CR rats had significantly higher $V_{TCA,N}$ and $V_{cyc(tot)}$ compared to the age-matched controls.