

# Caloric Restriction Impedes Age-related Decline of Neuronal Function and Energy Demand

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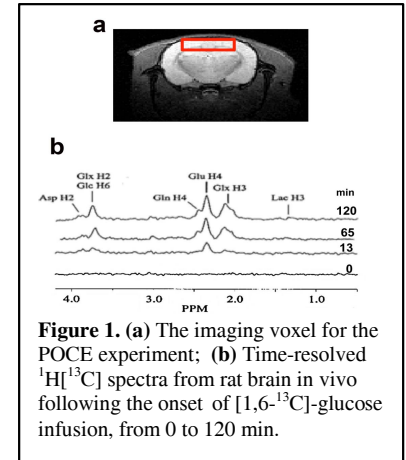
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**Target Audience:** The study will benefit general audience, clinicians and researchers who are interested in knowing the dietary effects on aging brain metabolism using state-of-the-art *in vivo* brain imaging techniques.

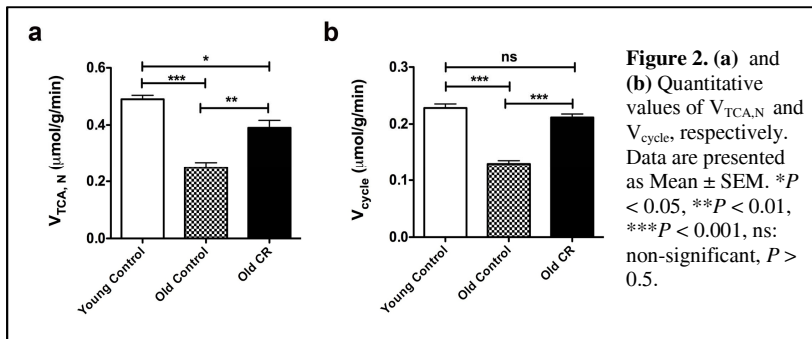
**Purpose:** Brain function plays a crucial role in lifespan determination<sup>1</sup>. Reduced brain metabolism has been reported in the aging brain and has been proposed to be a major factor in the loss of brain function with aging. Thus, preserving brain function and metabolism with age is critical 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, and to enhance memory in rodents<sup>2</sup>. However, the effects of calorie restriction on aging brain metabolism and neuronal activity remain largely unexplored. In the study, we asked whether CR can mitigate the bioenergetic and functional declines in aging brain. We used *in vivo* proton observed carbon edit (POCE) or <sup>1</sup>H[<sup>13</sup>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 ( $V_{cyc}$ ) in rats. We hypothesized that CR rats may preserve brain bioenergetics with aging.

**Methods:** Young control (5 mo), old control and old CR male rats (24 mo) were purchased from NIA. Animals were anesthetized with alpha chloralose. *In vivo* <sup>1</sup>H[<sup>13</sup>C] NMR study was performed on a Varian 11.7T MR system with POCE sequence. The POCE spectra were obtained from a localized volume (8x4x6 mm<sup>3</sup>) that covered cortex and hippocampus (Fig. 1a). [1,6-<sup>13</sup>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-<sup>13</sup>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 ( $V_{cyc}$ ). We used one-way, repeated measures ANOVA to determine the difference of the measured indices between the three groups. Post-hoc testing was performed by Newman-Keuls test.

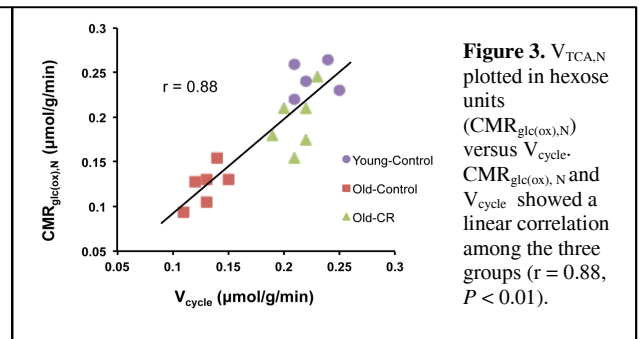
**Results:** Fig. 2 shows bar graphs of the rates of the neuronal TCA cycle ( $V_{TCA,N}$ ) and the glutamate glutamine cycle ( $V_{cyc}$ ) for each group. We found significant differences of  $V_{TCA,N}$  and  $V_{cyc}$  among the three groups ( $F = 27.9$ ,  $P < 0.001$ ). Notably, both  $V_{TCA,N}$  and  $V_{cyc}$  dramatically declined in normal aging rats. Compared to the young control rats, the old control rats had 51% ( $P < 0.001$ ) lower  $V_{TCA,N}$  (Fig. 2a) and 58% ( $P < 0.001$ ) lower  $V_{cyc}$  (Fig. 2b). Interestingly, the old rats treated with CR restored  $V_{TCA,N}$  and  $V_{cyc}$  by 44% ( $P < 0.01$ ; Fig. 2a) and 52% ( $P < 0.001$ ; Fig. 2b), respectively. Previously it has been found that when  $V_{TCA,N}$  is plotted in hexose units (i.e.,  $CMR_{glc(ox),N}$ ) against  $V_{cyc}$  that there is a close to 1:1 relationship between increments in glucose oxidation over an isoelectric baseline state and increments in glutamate-glutamine cycling, indicating a high energetic cost for neuronal function<sup>3</sup>. Fig. 3 shows a plot of  $V_{TCA,N}$  versus  $V_{cyc}$  for each of the studies, but where  $V_{TCA,N}$  is expressed in hexose units of  $CMR_{glc(ox),N}$ . The slope of the line showing the best fit is highly consistent with the previous findings<sup>3</sup>, thereby suggesting that the energetic costs of brain function remain similar with aging although and even with CR treatment.



**Figure 1.** (a) The imaging voxel for the POCE experiment; (b) Time-resolved <sup>1</sup>H[<sup>13</sup>C] spectra from rat brain *in vivo* following the onset of [1,6-<sup>13</sup>C]-glucose infusion, from 0 to 120 min.



**Figure 2.** (a) and (b) Quantitative values of  $V_{TCA,N}$  and  $V_{cyc}$ , respectively. Data are presented as Mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns: non-significant,  $P > 0.5$ .



**Figure 3.**  $V_{TCA,N}$  plotted in hexose units ( $CMR_{glc(ox),N}$ ) versus  $V_{cyc}$ .  $CMR_{glc(ox),N}$  and  $V_{cyc}$  showed a linear correlation among the three groups ( $r = 0.88$ ,  $P < 0.01$ ).

**Discussion:** Our results suggest that CR appears to be protective of mitochondrial function. The preserved mitochondrial function with age may reduce the generation of reactive oxygen species (ROS) and thus retard the cellular dysfunction and death through the lifespan. Our observations are also consistent with the theory of metabolic reserve as a determinant of cognitive aging. Metabolic reserve has been proposed as the ability of neuronal circuits to respond adaptively to perturbations in energy metabolism due to aging and disease processes, thereby maintaining their ability to support neuronal circuits and preventing declines in cognition. Old rats treated with CR have been previously shown to have enhanced memory compared to the age-matched controls<sup>2</sup>. Our findings provide direct evidence of preservation of cellular energy metabolism as proposed by this theory. The high cerebral metabolic reserve may also be due to the recruitment of additional fuel substrates for oxidative metabolism, such as ketone bodies. Increased ketone bodies level is associated with improved performance on learning and memory tests<sup>4</sup>. We hypothesize that ketone bodies may also play an important role in modulating brain metabolism and cognition in rats under CR. Future studies are needed to clarify this.

**Conclusion:** We have used MRS to demonstrate that during aging CR preserves mitochondrial energy production, energy demand and neuronal activity. These results provide a rationale for CR-induced sustenance of brain health with extended lifespan. Understanding of nutritional effects on brain function may have profound implications in human aging and other age-related neurodegenerative disorders.

**References:** 1. Mattson, M.P., Duan, W. & Maswood, N. How does the brain control lifespan? *Ageing Res Rev* 1, 155-165 (2002); 2. Carter, C.S., Leeuwenburgh, C., Daniels, M. & Foster, T.C. Influence of caloric restriction on measures of age-related cognitive decline: role of increased physical activity. *J Gerontol A Biol Sci Med Sci* 64, 850-859 (2009); 3. Hyder, F., Rothman, D.L. & Bennett, M.R. Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species and activity levels. *Proc Natl Acad Sci U S A* 110, 3549-3554 (2013); 4. Maalouf, M., Rho, J.M. & Mattson, M.P. The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Res Rev* 59, 293-315 (2009).