Preserved Brain Metabolism in Aging with Mitochondrial Mutation

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Introduction: Mitochondrial oxidative phosphorylation of glucose is the predominant mode of energy generation (ATP production) in the brain. Mitochondrial function declines with age and do so in an accelerated manner in neurodegenerative disorders such as Alzheimer's disease (1). Preserved mitochondrial integrity is thus generally presumed to be necessary for healthy aging. Paradoxically, several mitochondrial electron transport chain (ETC) mutations have been recently found to be associated with increased longevity in invertebrates and rodents (2,3). It is hypothesized that this increased lifespan is associated with augmented neural resilience (3) and the rate reduction in brain aging. However, the hypothesis has not been tested directly. Here we used multimodal imaging methods (e.g., PET and MRS) to determine brain metabolism in aging process of Surf1 knockout mice, which have 50-80% reduced mitochondrial complex IV ETC activity but display 21% increased median lifespan (3). The study demonstrated a significant correlation between glucose metabolic pathway-shifting and brain function preservation in aging.

Material and Methods: C57/Bl6J_DBA2 young (6-7 months of age, N = 6) and aged (17-18 months of age, N = 4) adult male Surf1 KO mice, and in age-, gender-, and genetic background-matched WT; ($Surf1^{+/+}$; N = 4 for both young and aged WT) were used in the study. The study protocol was approved by the IACUC of the UTHSCSA. Mice were imaged under 1.0-1.2% isoflurane. Respiration rate (90-130 bpm) and rectal temperature (37 ± 0.5°C) were continuously monitored. PET measurements were performed on a Focus 220 MicroPET scanner. ^{18}FDG of 0.5 mCi was injected through the tail vein. Emission data was acquired for 20 min after 40 min of injection. Glucose uptake was determined using the mean standardized uptake value (SUV_{mean}) equation. All MR experiments were performed on a Bruker 7T magnet. ^{1}H MRS was acquired with a point-resolved spectroscopy PRESS sequence. A single voxel (3.5 mm cube) was placed at the iso-center of the mouse brain (water linewidth =17 Hz). TR/TE=2500/140 ms; with (256 averages) and without (4 averages) VAPOR water suppression and outer volume suppression (OVS) techniques. Data were processed using Bruker TOPSPIN software. Lac (1.33 ppm), NAA (2.02 ppm) and Glu/Gln (2.43 ppm) concentrations were determined from the ratio of intergraded intensities of the metabolite and that of the non-suppressed water signal (e.g., [Lac]/[Water])[27]. ^{31}P MRS was acquired with a surface coil tuned to ^{31}P Larmor frequency (121.6 MHz) and a NSPECT sequence. Parameters: TR=4000 ms and 160 averages. [ATP] was determined from the ratio of integrated intensities of the ATP γ peak and PCr using TOPSPIN. All of the measured variables of the four groups of mice were compared with one-way ANOVA. Post-hoc testing was done by Newman-Keuls test.

Results: CMR_{Glc} declined with age in both Surf1 KO and WT mice. However, Surf1 KO mice, both young and aged, had significantly higher CMR_{Glc} relative to the age-matched WT mice (Figs. 1a and b). CMR_{Glc} in aged Surf1 KO mice was not different from that in young WT mice (Fig. 1b). Brain [Lac] was significantly higher in the young Surf1 KO mice than the young WT controls ($p < 0.001$) (Figs. 1e and f). Brain [Lac] levels did not significantly increase with age in the Surf1 KO mice ($p = 0.059$), but did increase significantly in the WT mice ($p < 0.001$). [NAA] and [Glu]/[Gln] decreased significantly with age in WT mice, but not in the Surf1 KO mice. Young Surf1 KO mice and young WT mice had similar [NAA] ($p = 0.13$; Fig. 1g) and [Glu]/[Gln] ($p = 0.061$; Fig. 1h). Both [NAA] and [Glu]/[Gln] remained stable in the Surf1 KO mice with age, but significantly decreased with age in the WT mice. [ATP] was measured using ^{31}P MRS (Fig. 4A). Similar to [NAA] and [Glu]/[Gln], [ATP] was not significantly different between the young Surf1 KO mice and WT mice ($p = 0.074$; Figs. 1c and d). [ATP] was maintained well with age in the Surf1 KO mice, but reduced with age in the WT (Fig. 1d).

Discussion and Conclusion: Here we showed that i) the aged Surf1 KO mice had similar "brain age" (i.e., preserved hemodynamics and metabolism) compared to the young WT; ii) Surf1 KO mice had significant increases in glucose metabolism; iii) their glucose metabolic pathway shifted toward anaerobic glycolysis (i.e., increased [Lac]). Increased glycolysis of glucose may make significant contributions to the increased neuronal resilience in Surf1 KO mice. For example, glycolysis fuels Na/K-ATPase pump in astrocytes for the transport of glutamate from the synaptic cleft into astrocytes. Failure to remove glutamate efficiently from the synapse is deleterious because excessive glutamate acts as an excitatory neurotoxin (4). This neuroprotective effect is consistent with the observation that Surf KO mice have high resistance to glutamate toxicity (3). As a group, Surf1 KO mice exhibit extended longevity and, as shown here, metabolic profile supporting neural resilience to aging. In conclusion, we demonstrated the potential association between mitochondrial mutation and brain metabolism in aging in vivo in the Surf1 KO mice brain. Non-invasive multimodal neuroimaging approach has the potential to provide quantitative physiology and phenotypes of brain neurological disorders in living animals as well as means to evaluate novel therapeutic strategies longitudinally. More importantly, this approach can be readily applied to clinical settings.

References: (1) Cunnane et al. *Nutrition* 27:3 (2011); (2) Kirchman et al., *Genetics* 152:179 (1999); (3) Dell'agnello et al*., Hum Mol Genet* 16:431 (2007); (4) Olney et al., *Curr Opin Pharmacol* 3:101(2003).

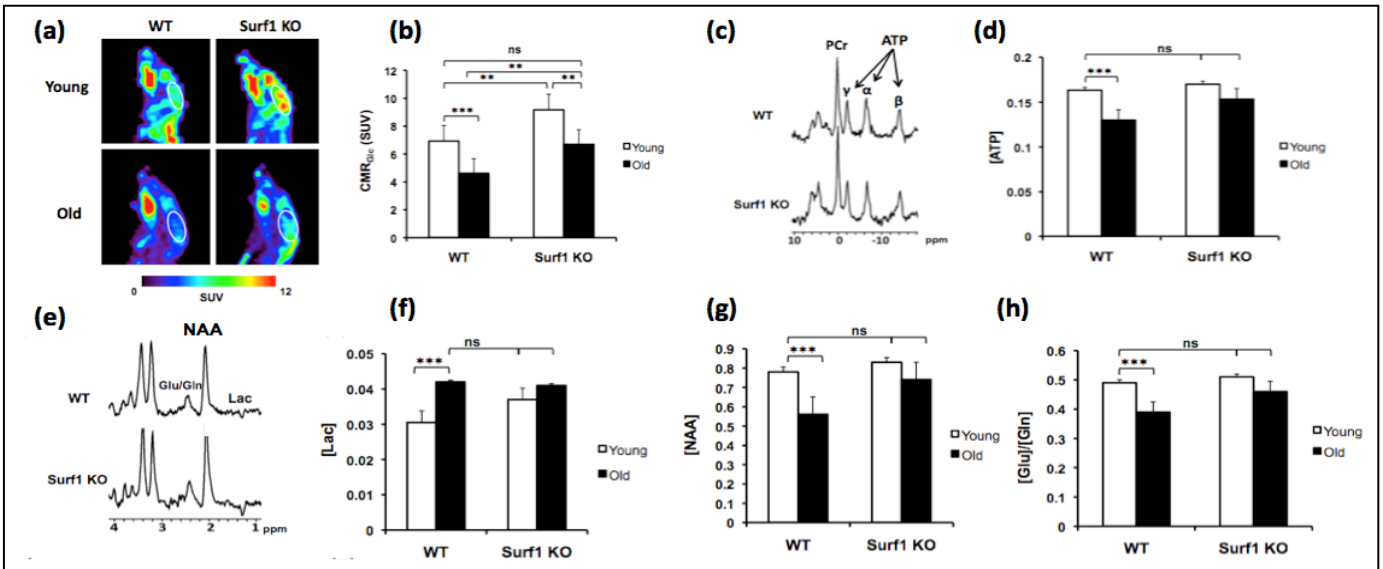

Figure 1. (a) Glucose uptake maps of a control and a Surf1 KO mice from each age group. Global CMR_{Glc} was taken from the ROI, circled by the white ovals. **(b)** Averaged CMR_{Glc} (mean ±SE) from each group. **(c)** ^{31}P MRS of a young control and a young Surf1 KO mice. **(d)** Averaged [ATP] (mean ±SE) from each group. **(e)** ^{1}H MRS of a young control and a young Surf1 KO mice. **(f)** Averaged [Lac]; **(g)** Averaged [NAA]; **(h)** Averaged [Glu]/[Gln] (mean ±SE) from each group.** $p < 0.05$; *** $p < 0.001$; ns: non-significant.