Elevated brain lactate measured by ^{1}H-MRS is an early phenotype due to mitochondrial dysfunction in the prematurely ageing mtDNA mutator mouse

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Purpose: Mitochondrial dysfunction may underlie ageing-related alterations in neuronal function and has been implicated in Alzheimer's and Parkinson's disease, as well as in stroke. The mitochondrial theory of ageing, first proposed by Harman in 1972, is based on the idea that damage to mitochondrial DNA (mtDNA) will slowly accumulate with time and cause ageing phenotypes by interfering with bioenergetic homeostasis or by loss of vital cells due to apoptosis and/or replicative senescence. The mitochondrial network in a mammalian cell contains ~10^{3}-10^{4} copies of mtDNA, which encodes key subunits of the respiratory chain enzyme complexes critical for maintaining cellular energy production. It has been extensively documented that the normal ageing process in humans, monkeys, and rodents is associated with accumulation of low levels of point mutations and deletions of mtDNA. A homozygous knock-in mouse expressing a proof-reading deficient version of the nucleus-encoded catalytic subunit (PolgA) of mtDNA polymerase has been developed to study the effects of progressive mitochondrial dysfunction, and shows many signs of premature ageing (Trifunovic *et al., Nature,* 2004). Mice have a 3- to 5- fold increase in levels of point mutations as well as increased levels of mtDNA deletions. This increase in somatic mtDNA mutations is associated with premature onset of human-like ageing-related phenotypes, including reduced life span, hearing loss, reduced fertility, weight loss, reduced subcutaneous fat, sarcopenia, enlarged heart, alopecia, anemia, kyphosis, and osteoporosis. In this study, we use ^{1}H-MRS to study metabolism *in vivo* in mtDNA mutator mice. We find abnormally high levels of lactate in the CNS using biochemical and functional assays.

Methods: ^{1}H-MRS was performed on cerebral cortex and striatum of mtDNA mutator mice (n=19, females) and wild-type littermate controls (n=18, females) and grouped into 6 age categories: 6-9 wks, 12-15 wks, 21-24 wks, 30-33 wks, 35-38 wks, and 40-42 wks. A horizontal 4.7 T/40 cm magnet (BioSpec Avance 47/40, Bruker, Ettlingen, Germany) equipped with a 12 cm inner diameter self-shielded gradient system (max. gradient strength 200 mTm^{-1}) was used. A linear birdcage resonator with an inner diameter of 25 mm was used for excitation and detection. Voxel shape and localization was achieved by point-resolved spectroscopy (PRESS) using Hermite radio frequency (RF) pulses with a matched bandwidth (BW) of 5 kHz. To enhance voxel shape definition, outer volume suppression (OVS) was applied using hyperbolic secant pulses with a BW of 20 kHz. As a result, the maximum voxel displacement referenced to lactate was about 10% of the voxel size. The volume of interest (VOI) for spectroscopy was chosen using a spin echo sequence with rapid acquisition and with relaxation enhancement (RARE) in axial, coronal, and sagittal planes with the following parameters: repetition time (TR)=3500 ms, echo time (TE)=15.9 ms, RARE factor=8 with RARE maximum=4, matrix size 256 x 256, slice thickness=1mm, and a field of view (FOV)=2.5mm. The volume of interest (VOI) in the cerebral cortex was 4 x 1.6 x 1.6 mm (10.2 µl), and was 5 x 2 x 2 mm (20 µl) in striatum. Anesthesia was induced and maintained during scans by spontaneous breathing of 1.7 – 2% isoflurane and 30% oxygen. Pulse was monitored and body temperature was kept at 36 ± 0.5°C. The quantification algorithm of LCModel™ applies linear combinations of model spectra to calculate the best fit of the experimental spectrum. Metabolite concentrations were calculated as ratios to the total creatine concentrations (Cr + PCr). Criteria for reliable metabolite quantification were based on the Cramér-Rao lower bounds (CRLB) for each metabolite, and those with CRLB less than 50% were considered for further analysis.

Results:

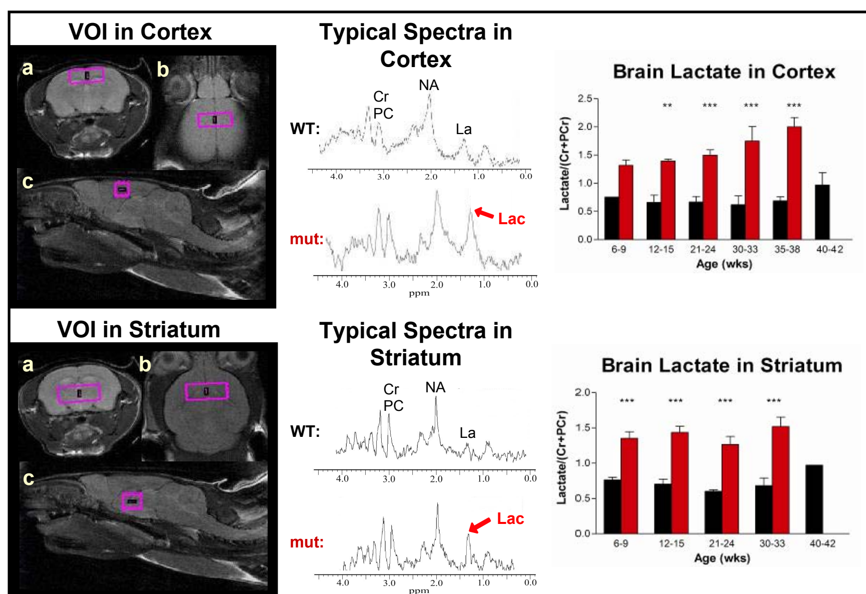

Figure 1. (left) Volume of interest (VOI) placement in (a) axial, (b) coronal, and (c) sagittal planes in cerebral cortex and striatum. (middle) Typical proton MR spectra observed in cerebral cortex (upper) and striatum (lower). (right) All mtDNA mutator mice (red, n=19) generating high quality spectra reflecting expected metabolites displayed a 2-fold increase in the lactate doublet peak occurring at 1.33 ppm in cerebral cortex and striatum, compared to wild-type littermates (black, n=18). Brain lactate levels were markedly increased throughout life from 6-9 weeks of age, Two-way ANOVA determined significances in mtDNA mutator mice in cerebral cortex ($F(1,25)=84.1$, $p<0.0001$) and in striatum ($F(1,24)=134$, $p<0.0001$). Bonferroni *post hoc* analyses to evaluate pairwise differences are denoted: ** $p<0.01$, and *** $p<0.0001$.

Conclusions: These findings demonstrate that a doubling of lactate levels in key brain areas is an early phenotype of the prematurely ageing mtDNA mutator mice and supports the hypothesis of abnormal metabolism in ageing due to mitochondrial dysfunction. Our findings also provide functional evidence for lactate as a marker in ageing and in illness. Furthermore, these data provide additional support for an alternative energy metabolism pathway in the brain. Finally, these findings demonstrate that early and chronic increases of CNS lactate levels are not associated with any obvious early behavioral disturbances.