Radiological Physics and Technology (2019) 12:232–233
https://doi.org/10.1007/s12194-019-00510-0

AUTHOR'S REPLY TO LETTER TO THE EDITOR

Early detection of elevated lactate levels in a mitochondrial disease model using chemical exchange saturation transfer (CEST) and magnetic resonance spectroscopy (MRS) with 7T MR imaging

Shigeyoshi Saito[1,2] · **Yusuke Takahashi**[3] · **Akiko Ohki**[2] · **Yasunori Shintani**[4] · **Takahiro Higuchi**[2,5,6]

Received: 28 February 2019 / Revised: 27 March 2019 / Accepted: 27 March 2019 / Published online: 1 April 2019

Keywords Ndufs4 knockout · Leigh syndrome · Chemical exchange saturation transfer · Magnetic resonance spectroscopy

To the Editor,

We appreciate the letter in response to our article [1]. Our report revealed that chemical exchange saturation transfer (CEST) and magnetic resonance spectroscopy (MRS) using 7T magnetic resonance imaging (MRI) could detect lactate level elevation prior to the formation of cerebral morphological abnormalities in Ndufs4 knockout (KO) mice, a mouse model of Leigh syndrome [1].

The letter claimed that Ndufs4 KO mice investigated in the present study do not represent an animal model of Leigh syndrome. We performed MRI and CEST in the mice when they were 6 weeks of age. They did not show any lesions in T_2-weighted MRI (T_2WI), but at around 8 weeks of age they manifested Leigh syndrome features with 100% penetrance. Ndufs4 KO mice that we used in this study were produced and well established as a model of Leigh syndrome by the Palmiter Laboratory at the University of Washington [2, 3]. A model of Leigh syndrome is characterized by failure to thrive, generalized hypotonia, developmental delay, central respiratory compromise, ataxia, and epilepsy [4], as well as bilateral and symmetrical necrotic lesions in the basal ganglia and brainstem, which appear as hyperintense lesions on T_2WI [5]. All the features were reproduced in the environment in our animal facility. Therefore, we conclude that Ndufs4 KO mice are an appropriate animal model of Leigh syndrome. The point we claim is that the increase in cerebral lactate level on MRS reflects an early stage of the disease before morphological abnormalities are detectable on T_2WI.

Actually, in our previous study, we performed serial measurement of brain MRI and MRS. Brain MRS was performed in the Ndufs4 KO mice and control mice at age 5–9 weeks using 7T MRI. In KO mice that survived until 9 weeks of age, both MRS and T_2WI were longitudinally performed in the same individuals at 5, 7, and 9 weeks. Brain MRS demonstrated increased lactate levels in KO mice than in control mice. The increased intracerebral lactate levels could be observed at 5 weeks of age, whereas no obvious abnormal findings were detected in T_2WI (Fig. 1a). Longitudinal MRS experiments revealed a temporal increase of intracerebral lactate levels, which peaked at week 7, followed by a decrease at week 9. During this period, further disease progression with brain lesions was detected on T_2WI [5]. Serial T_2WI of the brain revealed no abnormal lesions in KO mice at 5 weeks of age (Fig. 1a). However, at 9 weeks of age, bilateral and symmetrical lesions were detected in the postlateral portion of the brainstem in the KO mice only.

Our non-invasive imaging of cerebral lactate using CEST and MRS is useful for diagnosing some of mitochondrial disorders; however, there are mitochondrial

This reply refers to the article available at https://doi.org/10.1007/s12194-019-00511-z.

✉ Shigeyoshi Saito
saito@sahs.med.osaka-u.ac.jp

1 Division of Health Sciences, Department of Medical Physics and Engineering, Osaka University Graduate School of Medicine, Suita, Osaka 560-0871, Japan

2 Department of Biomedical Imaging, National Cardiovascular and Cerebral Research Center, Suita, Osaka 565-8565, Japan

3 Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

4 Department of Medical Biochemistry, Osaka University Graduate School of Frontier Bioscience, Suita, Osaka 565-0871, Japan

5 Comprehensive Heart Failure Center, University of Wuerzburg, Würzburg 97078, Germany

6 Department of Nuclear Medicine, University of Wuerzburg, Würzburg 97078, Germany

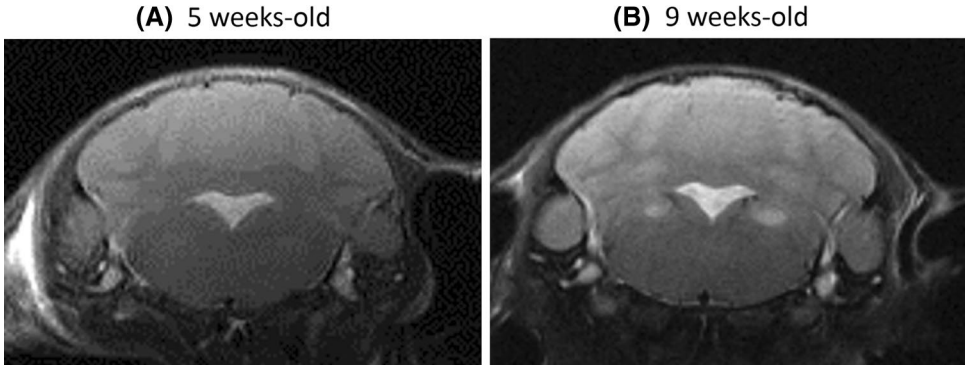

Fig. 1 Representative T_2W image of KO mouse brain. **a** An image at 5 weeks of age. **b** An image at 9 weeks of age showing hyperintense lesions located in the postlateral portion of the brainstem (*T_2WI* T_2-weighted, *KO* knockout)

disorders without cerebral involvement or mitochondrial disorders without elevation of cerebral lactate levels, as Finsterer pointed out. However, it should be noted that assessment of intracerebral lactate levels using ^{1}H MRS is the most widely used and possibly useful method to evaluate mitochondrial disease activity in clinical settings [6, 7]. In addition, we used brain proton MRS in Ndufs4 KO mice to investigate whether they were appropriate animal models of Leigh syndrome by monitoring their intracerebral lactate levels as a biomarker of mitochondrial disease progression [5].

The letter said that we should discuss why MTR asymmetry was mapped at offset frequencies of 3.0 or 3.5 ppm. As we discussed in previous reports [1], it is necessary to carefully consider the effects of nuclear Overhauser enhancement on CEST around 3.5 ppm. There was no alteration of glutamate in the homozygous Ndufs4 KO mice. This suggests that the CEST signal from glutamate at around 3.0 ppm and APT at around 3.5 ppm did not affect glutamate levels or other metabolites in the KO mice.

We performed MRI at the same temperature because CEST imaging may be temperature dependent [1]. In the Materials and methods section of our reports, body temperature was reported to be maintained at 36.5 °C with regulated water flow and continuously monitored using a physiological monitoring system (SA Instruments, Inc., Stony Brook, NY, USA) [1, 5]. In addition, MRI was performed with the mice under general anesthesia with isoflurane (2.0% for induction and 1.0% for maintenance for the KO mice due to anesthesia sensitivity, and 3.0% for induction and 2.0% for maintenance for the control mice) [1, 5].

We conclude that an early age of Ndufs4 KO mouse shows disorders in bioenergetics that are manifested by elevated lactate levels. The usefulness of brain CEST and ^{1}H MRS in mitochondrial disorders will need to be further explored in the future.

Funding This work was supported by Grants-in-Aid for Scientific Research (Kakenhi, Nos. 16K090170 and 15K21774) from the Japan Society for the Promotion of Science (JSPS).

Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest to report.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

1. Saito S, Takahashi Y, Ohki A, Shintani Y, Higuchi T. Early detection of elevated lactate levels in a mitochondrial disease model using chemical exchange saturation transfer (CEST) and magnetic resonance spectroscopy (MRS) at 7T-MRI. Radiol Phys Technol. 2019;12(1):46–54.
2. Kruse SE, Watt WC, Marcinek DJ, Kapur RP, Schenkman KA, Palmiter RD. Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. Cell Metab. 2008;7(4):312–20.
3. Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD. Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proc Natl Acad Sci USA. 2010;107(24):10996–1001.
4. Finsterer J. Leigh and Leigh-like syndrome in children and adults. Pediatr Neurol. 2008;39(4):223–35.
5. Takahashi Y, Kioka H, Shintani Y, Ohki A, Takashima S, Sakata Y, Higuchi T, Saito S. Detection of increased intracerebral lactate in a mouse model of Leigh syndrome using proton MR spectroscopy. Magn Reson Imaging. 2019;58:38–43.
6. Kaufmann P, Shungu DC, Sano MC, Jhung S, Engelstad K, Mitsis E, Mao X, Shanske S, Hirano M, DiMauro S, De Vivo DC. Cerebral lactic acidosis correlates with neurological impairment in MELAS. Neurology. 2004;62(8):1297–302.
7. Boddaert N, Romano S, Funalot B, Rio M, Sarzi E, Lebre AS, Bahi-Buisson N, Valayannopoulos V, Desguerre I, Seidenwurm D, Brunelle F, Brami-Zylberberg F, Rotig A, Munnich A, de Lonlay P. 1H MRS spectroscopy evidence of cerebellar high lactate in mitochondrial respiratory chain deficiency. Mol Genet Metab. 2008;93(1):85–8.