www.nature.com/pr

Pediatric RESEARCH

COMMENT

Reply to comment: "Brain creatine alteration and executive function deficits in children born very preterm"

Barbara Schnider[1,2], Vera Disselhoff[1,2], Beatrice Latal[2,3], Flavia Wehrle[1,2,3], Cornelia Hagmann[1,2] and Ruth Tuura[2,4]

Pediatric Research (2021) 90:256–258; https://doi.org/10.1038/s41390-020-01281-w

Magnetic resonance spectroscopy (MRS) provides a powerful, in vivo method for evaluating the metabolic profile of the brain or other organs, noninvasively. Metabolite levels can be estimated from the size of the characteristic peaks (or sets of peaks) for each metabolite, but in order to derive quantitative estimates of the metabolite levels, it is necessary to calibrate the signal from each metabolite against that from a reference standard. A number of calibration methods have been suggested for MRS, but the two most widely used methods involve scaling either to the unsuppressed water peak or to the signal arising from the Creatine peak.

There is an ongoing debate about the best calibration standard to use for quantitative MRS, but each has its merits, and it may be the case that different scaling methods might be preferred to address different clinical or research questions. In the present response to the commentary by Ostojic,[1] we aim to discuss the relative merits of each method within the context of pediatric MRS studies, and particularly of our study published recently in *Pediatric Research* entitled "Altered brain metabolism contributes to executive function deficits in school-aged children born very preterm."[2]

From a technical standpoint, the localization methods used for the Point RESolved Spectroscopy (PRESS) MRS sequence cause a chemical shift displacement (CSD) error, whereby the signals from different metabolites arise from different locations, shifted by a certain distance from the prescribed voxel. In practice, only the signal from one specific metabolite (typically *N*-acetyl aspartate (NAA) or sometimes an intermediate frequency between NAA and Creatine) originates from the intended location of the selected voxel of interest, as displayed on the magnetic resonance imaging (MRI) console. The signal for all other metabolites is displaced by a degree dependent on the difference in the spectral frequency of each metabolite from the reference frequency (e.g., of NAA or between NAA and Creatine). A recent methodological consensus paper from the Institute for Magnetic Resonance in Medicine (ISMRM) MRS study group discusses this issue in detail,[3] and Fig. 2 of this consensus paper shows an example of the CSD error in practice. Since the chemical shift of water (4.7 ppm) is further removed from the chemical shift of most of the other neurometabolites, Creatine scaling is typically associated with a smaller CSD error than water scaling, for most metabolites apart from myo-inositol, whose multiple peaks lie between the Cr and water peaks but are slightly closer to the water peak. The CSD error increases with increasing magnetic field strength and is therefore more prominent at 3 T than at 1.5 T but is considerably improved with more recent MRS pulse sequences like the semi-Laser sequence.[3]

In addition to the reduced CSD error for most metabolites, one advantage of Creatine scaling is that the Creatine signal originates from the same (water-suppressed) metabolite subspectra as the other neuro-metabolites, and the data are therefore acquired contemporaneously, while the unsuppressed water signal is either acquired in a separate scan or at the beginning or end of the MRS acquisition.[4] Subject motion may therefore cause the water signal to be acquired from a different location, although it can also degrade the spectral quality overall, depending on the degree and duration of motion and when it occurs. For pediatric populations prone to motion during the scan, Creatine scaling is less sensitive to motion between the water-suppressed subspectra (the metabolite lines) and the water lines. However, a disadvantage of Creatine scaling in comparison to water scaling is that the Creatine signal is considerably smaller than the water signal (by a factor of 10,000) and therefore shows higher variability due to its lower signal-to-noise ratio. Due to the lower variability of the water signal, water-scaled concentrations can be more reproducible and hence are sensitive to more subtle changes.

Another advantage of water scaling, as discussed in the commentary by Ostojic,[1] is that it removes ambiguity in ascribing observed changes to the metabolite of interest (in the numerator of the Creatine ratio) or to the effects of Creatine in the denominator. Previous studies in both adults and children have shown that, while Creatine is arguably the most stable of the neurometabolites, it demonstrates significant changes with development and in the presence of pathology.[5] This is an important point affecting MRS studies in pediatric patient groups and should be considered carefully when interpreting the results. As discussed in the commentary by Ostojic,[1] previous studies have shown specific alterations in Creatine metabolism in prematurity, so it is possible that the Creatine signal may be altered in children and adolescents born very preterm. However, it is important to note that water-scaled concentrations are also effectively ratios to the water signal, which also shows developmental changes, as both the brain water concentration and the relaxation times change with age,[6–9] and the unsuppressed water signal measured with MRS is sensitive to both of these effects.[10] In addition to showing developmental effects, the relaxation times can also show persistent differences in children and adolescents born very preterm. In a previous study, we observed significantly increased

[1]Department of Neonatology and Pediatric Intensive Care, University Children's Hospital Zurich, Zurich, Switzerland; [2]Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland; [3]Child Development Center, University Children's Hospital Zurich, Zurich, Switzerland and [4]Centre for MR Research, University Children's Hospital Zurich, Zurich, Switzerland
Correspondence: Ruth Tuura (ruth.tuura@kispi.uzh.ch)

Received: 16 September 2020 Accepted: 23 September 2020
Published online: 19 November 2020

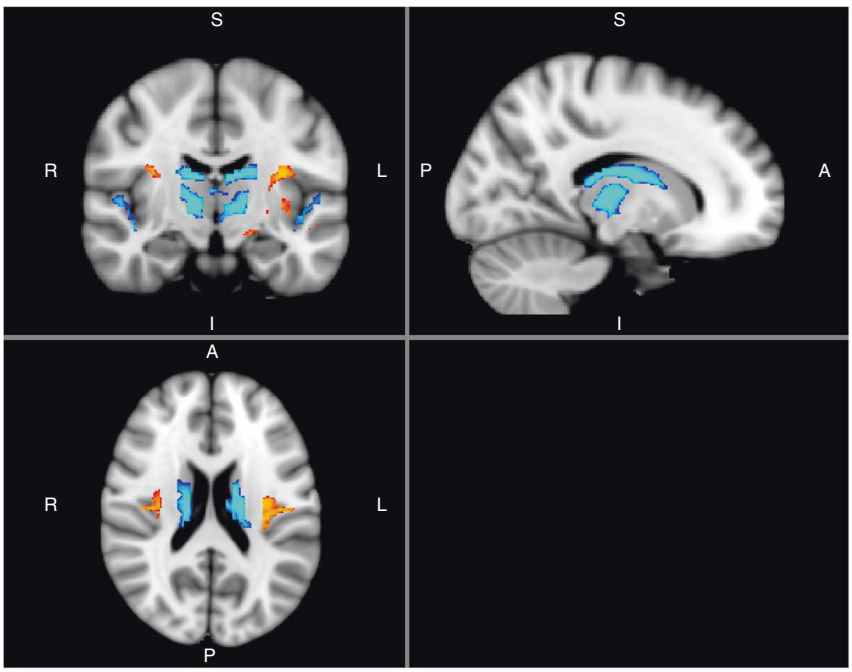

Fig. 1 In a group of 31 very preterm participants (born prior to 32 weeks of gestation, with no evidence of leukomalacia or hemorrhagic infarction, no diagnosis of cerebral palsy, and IQ in the normal range (>85)), T1 relaxation times measured with quantitative MR relaxometry were significantly increased in the basal ganglia and thalamus (regions depicted in blue) and decreased in the insula and amygdala/hippocampus (regions depicted in red). Data are shown with a statistical threshold of $p < 0.05$, corrected for multiple comparisons). The control group consisted of 31 healthy term-born participants matched to the very preterm participants by age and sex.

T1 relaxation times in a number of brain regions including the basal ganglia (one of the regions examined in our recent paper) in an independent cohort of adolescents born very preterm (mean age 12 years, range 10–16) when compared to an age-matched healthy control group (see Fig. 1). Specifically, the T1 relaxation times were increased by 32 and 36% in the left and right caudate, respectively, and were also correlated with executive function measures.[11] While this study measured the MR-visible T1 relaxation time in tissue, the tissue T2 relaxation times have also been reported to alter with development, and with prematurity.[5,12,13] While pathological changes in Creatine could confound the interpretation of Creatine-scaled metabolite ratios, such a pronounced change in water relaxation time could also induce a bias in water-scaled concentrations derived with MRS, depending on the exact parameters of the MRS protocol (and particularly the repetition time and echo time used for the acquisition, since these will affect the degree to which T1 and T2 relaxation times affect the water signal).

Therefore, both Creatine and water have been reported to show developmental and pathological changes, which should be considered carefully when interpreting results from pediatric MRS studies. One way to ascertain whether significant results are likely to arise from the numerator (the metabolite of interest) or the denominator (either water or Creatine) is to check the results for other metabolites in the same cohort: if all metabolite ratios to Creatine or water go in the same direction (e.g., are increased in a particular patient group or show the same direction of correlation with a behavioral or clinical measure), then it may be the case that the observed changes are driven by changes in the denominator rather than the numerator. In our recent paper in *Pediatric Research*,[2] the apparent correlations between frontal Glutamate + Glutamine (Glx) and frontal myo-inositol (mI) (both referenced to Creatine (Cr)) went in different directions and are therefore unlikely to be driven by Creatine. In his commentary, Ostojic[1] pointed out that, if the results were obtained from different brain regions, they could still be confounded by Creatine changes if Creatine showed a significant but opposite change in these two regions. To address this point, we can clarify that the significant results were observed only within the frontal MRS voxel, and not within the basal ganglia voxel. We have also performed an additional analysis, calculating water-scaled metabolite concentrations, corrected for cerebrospinal fluid (CSF) contamination within the voxel, and comparing these both on a groupwise basis and in relation to executive function measures. In our cohort, neither significant group differences in Cr/H_2O (frontal: $t = 0.28$, $p = 0.78$, basal ganglia: $t = 0.85$, $p = 0.40$) nor an association between Cr/H_2O and the global executive function score were observed when adjusting for age at assessment, sex, socioeconomic status, and processing speed (frontal: overall model: $F(6,91) = 17.76$, $p < 0.001$, adjusted $R^2 = 0.51$; Cr/H_2O: $B = 0.003$, 95% confidence interval (CI) [−0.15, 0.16], $\beta = 0.003$, $p = 0.97$; basal ganglia: overall model: $F(6,87) = 16.78$, $p < 0.001$, adjusted $R^2 = 0.51$; Cr/H_2O: $B =$ -0.01, 95% CI [−0.10, 0.07], $\beta = -0.03$, $p =$ 0.74). The significant association between frontal Glx/Cr and executive function reported in our paper remained significant when referencing to the unsuppressed water peak (association between Glx/H_2O and the global executive function score: overall model: $F(6,89) = 19.11$, $p < 0.001$, adjusted $R^2 = 0.53$; Glx/H_2O: $B =$ 0.08, 95% CI [0.01, 0.14], $\beta = 0.18$, $p = 0.02$). The association between frontal mI/Cr and the global executive function score became nonsignificant after referencing to water (overall model: $F(6,91) = 18.13$, $p < 0.001$), adjusted $R^2 = 0.51$, mI/H_2O: $B = -0.07$, 95% CI [−0.22, 0.07], $\beta = -0.07$, $p = 0.32$. All regression models were adjusted for the covariates mentioned above).

For studies in older children and adolescents, some of the advantages of Creatine scaling (e.g., the lower CSD error) may become less important when the current standard PRESS sequence is replaced by more modern sequences like the semi-Laser sequence, but other advantages (e.g., the lower sensitivity to motion between the acquisition of the water and metabolite lines) may still be a consideration when selecting the scaling method of choice for a given study. However, in the studies of neonates the

advantages of Creatine scaling are arguably more prominent: the CSD error exerts a greater effect in neonates due to the smaller brain size, and the corrections for the tissue composition (e.g., the fractional volumes of gray matter, white matter, and CSF) within the voxel are currently challenging to calculate given a lack of widely available, validated software for segmentation of neonatal brain MRI. Creatine ratios (either concentration ratios or peak area ratios) are therefore far more common in the neonatal MRS literature than water-scaled concentrations. For our recently published study,[2] we opted to calculate ratios to Creatine partly for consistency with the neonatal literature, enabling a more direct comparisons to be drawn between our observations in school-aged children born very preterm and previous studies in premature infants. Such comparisons can be useful for assessing whether our observations represent persistent changes or alterations in the developmental trajectory of brain metabolism. However, we are aware of the limitations of Creatine scaling, and we welcome the opportunity to discuss these points in more detail within the scope of this response.

For developmental MRS studies, given the maturational and pathological changes that can affect both the Creatine and water signals, reporting both sets of ratios may be advantageous since it would allow the reader to assess the specificity of the findings with regard to the numerator or the denominator of the respective ratios. However, reporting both sets of results may also bring statistical challenges, particularly regarding the correction for multiple comparisons, since the ratios to Creatine and water are not statistically independent. For the reasons discussed in this response, both scaling methods have their merits, but both also have disadvantages that should be considered carefully when interpreting apparent changes in metabolite levels in the presence of pathology or in the context of brain maturation.

ACKNOWLEDGEMENTS

This project is supported by the Swiss National Science Foundation (grant number 320030_169733), the Gottfried and Julia Bangerter-Rhyner Foundation, the Hartmann Müller Foundation, the Anna Mueller Grocholski Foundation, and the Uniscientia Foundation.

AUTHOR CONTRIBUTIONS

All listed authors are aware that they are responsible for the reported research. They have participated in the conception and design of the original research, the analysis and interpretation of the data, the drafting or revising of the reply, and they have approved the final reply as submitted.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Ethics statement: The study was approved by the local ethical committee. Written informed consent was obtained from a parent as well as from participants aged >15 years. Younger participants provided oral consent.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Ostojic, S. M. Brain creatine alteration and executive function deficits in children born very preterm. *Pediatr. Res.* **88**, 704 (2020).
2. Schnider, B. et al. Altered brain metabolism contributes to executive function deficits in school-aged children born very preterm. *Pediatr. Res.* **88**, 739–748 (2020).
3. Wilson, M. et al. Spectroscopy of the brain: review and recommendations. *Magn. Reson. Med.* **82**, 527–550 (2020).
4. Mullins, P. G. et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *Neuroimage* **86**, 43–52 (2014).
5. Dezortova, M. & Hajek, M. 1H MR spectroscopy in pediatrics. *Eur. J. Radiol.* **67**, 240–249 (2008).
6. Lee, S. M. et al. Age-related changes in tissue value properties in children. *Investig. Radiol.* **53**, 236–245 (2018).
7. Eminian, S., Hajdu, S. D., Meuli, R. A., Maeder, P. & Hagmann, P. Rapid high resolution T1 mapping as a marker of brain development: normative ranges in key regions of interest. *PLoS ONE* **13**, 1–16 (2018).
8. Deoni, S. C. L., Dean, D. C., Remer, J., Dirks, H. & O'Muircheartaigh, J. Cortical maturation and myelination in healthy toddlers and young children. *Neuroimage* **115**, 147–161 (2015).
9. Deoni, S. C. L., Dean, D. C., O'Muircheartaigh, J., Dirks, H. & Jerskey, B. A. Investigating white matter development in infancy and early childhood using myelin water faction and relaxation time mapping. *Neuroimage* **63**, 1038–1053 (2012).
10. Gasparovic, C. et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn. Reson. Med.* **55**, 1219–1226 (2006).
11. O'Gorman, R. L. et al. Quantitative MR relaxometry reveals subcortical T1 differences in very preterm children and adolescents. In *24th Annual Meeting of the Society for Magnetic Resonance in Medicine* 1404 (ISMRM, 2016).
12. Counsell, S. J. et al. T2 relaxation values in the developing preterm brain. *Am. J. Neuroradiol.* **24**, 1654–1660 (2003).
13. Robertson, N. J. & Cox, I. J. In *MRI of the Neonatal Brain* (ed. Rutherford, M. A.) (Saunders Ltd., 2002).