

AWARD NUMBER: W81XWH-18-1-0797

TITLE: Early Detection of Alzheimer's Disease by Relayed NOE CEST MRI

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CONTRACTING ORGANIZATION:

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14. ABSTRACT We evaluated a new chemical exchange saturation transfer (CEST) technique, relayed nuclear Overhauser effect (rNOE)-CEST, which is able to detect the protein aggregation in Alzheimer's Disease (AD) mouse that associated with the Aβ accumulation. One optimized ultra short echo (UTE)-CEST method was applied on APP AD mouse models for detecting rNOE-CEST with high sensitivity and robust to motions. Strong age dependent rNOE-CEST signal was found in the APP mice compared to the wild type mouse through our longitudinal study. In order to make the UTE-CEST sequence available for clinical application, we further improved the method and introduced the Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction (PROPELLER) based method for the rapid acquisition of multi-slice rNOE-CEST on 3T human scanner					
15. SUBJECT TERMS MRI, Alzheimer's Disease, CEST, relayed NOE					
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Alzheimer's disease (AD) is the most common cause of dementia, and currently, more than 5 million people have AD in the US alone. The accumulation of neuritic plaques comprised of amyloid-beta (A β) peptides and neurofibrillary tangles (NFT) of hyperphosphorylated tau are the major histopathological hallmarks of Alzheimer's disease. Therefore, the detection of the aggregated proteins involved in AD is expected to be a promising strategy for the early diagnosis of the disease. We recently developed a new chemical exchange saturation transfer (CEST) technique, UTE-CEST, which is able to obtain high-resolution rNOE-CEST MRI insensitive to motion. We hypothesize that the rNOE-CEST signal detected by CEST-UTE on AD mouse is associated with the A β and NFT accumulation due to the line broadening of amide and aliphatic proton signal in proteins introduced by protein aggregation. The optimized UTE-CEST method will be applied on two AD mouse models to verify that the technique is sensitive enough to detect the early protein aggregation seen in AD disease. Upon the successful completion of this proposal, we anticipate developing a new clinic-ready MRI technique that detect and evaluate the AD associated protein aggregation.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Alzheimer's Disease, plaque, amyloid-beta (A β), MRI, ultra-short echo time (UTE), chemical exchange saturation transfer (CEST), Nuclear Overhauser effect (NOE)

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. To optimize and evaluate the MRI signal sensitivity and specificity of rNOE-CEST MRI techniques for detecting A β and NFT accumulation in mouse brain.

Major Task 1: A UTE-CEST sequence will be developed to acquire high-resolution rNOE-CEST CEST on an animal MRI scanner.

This task was completed in Apr. 30, 2019.

Major Task 2: Determine the sensitivity of protein CEST in detecting the protein aggregation on phantoms and two AD mouse models.

This task was completed by 100% before Oct. 20, 2019.

Aim 2. To verify the early detection of A β and NFT pathology in AD mouse by rNOE-CEST MRI.

Major Task 1: The optimized multi-slice UTE-CEST method will be applied on the APP and Tau AD mouse models for a longitudinal study (1.5 year) to verify that the technique is sensitive enough to detect the early protein aggregation occurring in AD brain. The individual measures of metabolism by *in vivo* MRS and quantitative MT (qMT) in each animal for each stage of disease will be performed to examine the CEST contributions from the metabolites and macromolecules. This task started from May. 1 and was completed by 60% before Oct. 30, 2020.

Major Task 2: In order to monitor the initial stage of the protein aggregation, the histological analysis will be performed at four time points over the period of one year (4, 6, 8 and 10 month). After the final MRI experiments (18 month), all animals will be scarified for histological analysis to confirm the burden and the distribution of the A β and NFT in brain. This task was performed in parallel to task 1 and was completed by 30% before Oct. 30, 2020.

Aim 3. Implementation and optimization of the UTE-CEST sequence on a 3T clinical scanner

Major Task 1: The UTE-CEST sequence will be programed on the Philips Ingenia 3T Scanner. The width and power of the saturation pulses in the UTE-CEST will be optimized on a water phantom and several BSA phantoms to minimize the water direct saturation and MTC contaminations.

This task was performed from Jan1. 2020 and was completed.

Major Task 2: Developing an image acquisition and post-processing pipeline for the correction of the B0 and B1 inhomogeneity on the 3T scanners when applying UTE-CEST sequence. This task is in progress.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) major activities

Longitudinal Study on AD mouse models and Clinical translation of the method:

During this reporting cycle, we continue worked on the longitudinal study on AD animals in the first cohort (last time point 12 month) with optimized UTE-CEST sequence and begin to perform the longitudinal study on the second batch of APP (n=3) and WT(n=3) mice and begin to perform the longitudinal study. we scanned the one more time point (12 month) for the three mouse models with optimized UTE-CEST sequence. Histological analysis was performed on WT and APP mice with same age as those performed with MRI.

While waiting for the more cohorts of the mice, we begin to work on the translation of the technique on human scanner and begin the pulse programming on Philips 3T MRI scanner. Due to the limited acquisition speed of the UTE-CEST based radial readout method, we applied the Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction (PROPELLER) based method for the rapid acquisition of multi-slice CEST on 3T human scanner. Figure 1a shows the steady-state radial sampling CEST sequence based on the PROPELLER sequence. Every TR contains a sinc-gauss saturation pulse followed by a train of gradient echo detection ($TR/TE/\text{flip angle} = 10 \text{ ms}/3\text{ms}/10^\circ$), which generates the blade lines in one blade. Previous pulsed CEST study already demonstrated that one pulse width larger than 40 ms will achieve enough frequency selectivity at 3T. Hence, the pulse width of the saturation pulses is set to 50 ms and the peak B1 values vary from $0.8 \mu\text{T}$ to $1.5 \mu\text{T}$. The oversampling rate is 150% for PROPELLER method, while 8 blade lines in one blade were collected. The time for the multi-slice acquisition was 7.6 s per irradiation frequency. We programed Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction (PROPELLER)-CEST on Philips 3T Elition MRI scanner. We tested the sequence using phantoms with the cross-linked bovine serum albumin (BSA) and BSA solutions. Three phantoms were prepared, a 10% cross-linked BSA (10%crossBSA), 10% BSA solution (10% BSA) and a creatine (50 mM) solution mixed with 10% cross-linked BSA (Cr+10%crossBSA). BSA was utilized to mimic the strong background signal commonly seen in *in vivo* tissue, while the creatine guanidinium protons at 2 ppm will be used to mimic the amide and aliphatic protons seen in tissues. BSA crosslinking was achieved using 25 $\mu\text{L}/\text{mL}$ glutaraldehyde solution. Phantoms were prepared in phosphate buffered saline (PBS), titrated to pH 7.3.

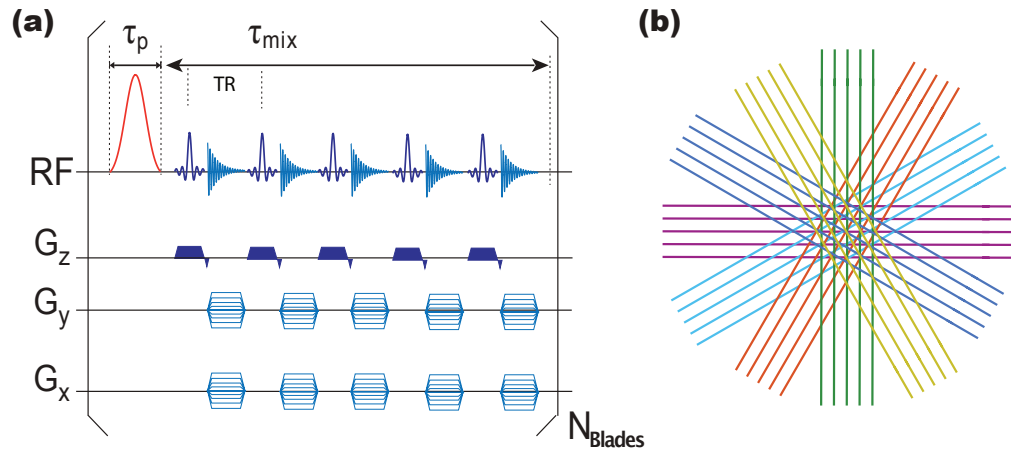


Figure 1: (A) Timing diagram of the PROPELLER-CEST sequence. τ_p is the duration of the saturation pulses, while τ_{mix} is the mixing time for saturation transfer between successive saturation pulses. (B) illustration of the sampling of k-space in PROPELLER-CEST. The k-space was acquired using a set of radially directed blades. Each blade is composed of multiple parallel phase-encoded lines that is collected using gradient echo methods. In this illustration and the sequence in Fig. A, five blade lines are acquired in a single shot.

2) *specific objectives*

The UTE-CEST and PROPELLER-CEST sequence will be programed on the Philips Ingenia 3T Scanner. The Creatine and BSA phantoms for 3T clinical scanners will be prepared for the following optimization. Developing an image acquisition and post-processing pipeline for the correction of the B0 and B1 inhomogeneity on the 3T scanners when applying UTE-CEST and PROPELLER-CEST sequences.

3) *significant results or key outcomes*

Immunohistochemical analysis on WT and APP mouse brain (n=3). Antibodies specific to human A β (6E10: 1:1,000; SIG-39300, Covance) to monitor plaque accumulation, as well as antibodies specific to endogenous phosphorylated S422 of tau (pS422: 1:2,000; 44764G, Invitrogen, Carlsbad, CA) for examining tangle formation were performed. Typical immunohistochemical analysis results for the cortex region of the WT and APP mouse brains are plotted in Fig. 2. At this age, the plaques are already abundant in APP mice, while not yet detectable in Tau and WT mice (Fig. 2a). Tau tangles are still very rare at this age (Fig. 2b), which is consistent with a previous observation that widespread tangles occur after 9 months.

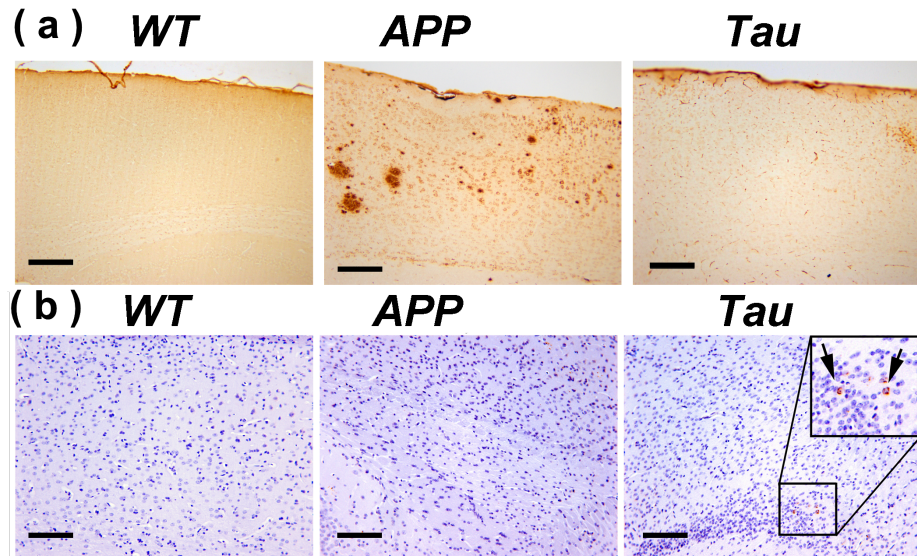


Figure 2. Brain sections of WT, APP and Tau mice stained by antibodies specific to A β (6E10) (a), phosphorylated tau: Tau-pS422 (b). Scale bars are 200 μ m.

The rNOE-CEST MRI was performed using the multi-slice UTE-CEST (msUTE-CEST) optimized in the previous grant periods. The following images show the comparison between the two types of mice for the rNOE values in the cortex region (updated with new number of animals). It can be seen that a clearly difference between WT and APP mice is observed at month 12 (5.2% vs 4.6%). Strong age dependent rNOE-CEST signal was also found in the APP mice as indicated from the longitudinal study (cortex).

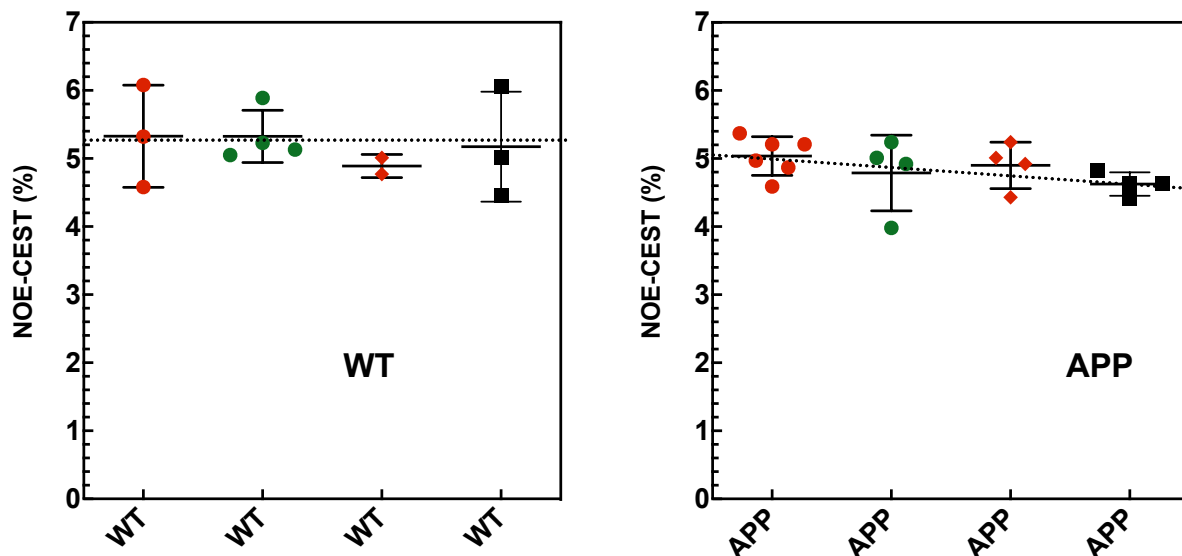


Figure 3. NOE-CEST signal from brain cortex as a function of age for WT and APP mice.

Below two images demonstrated the optimization results of the PROPELLER-CEST on the 3T MRI with different pulse number and number Blades. It can be seen that the maximum CEST signal was achieved at around 0.8 μ T and the signal is not sensitive to the number of blades.

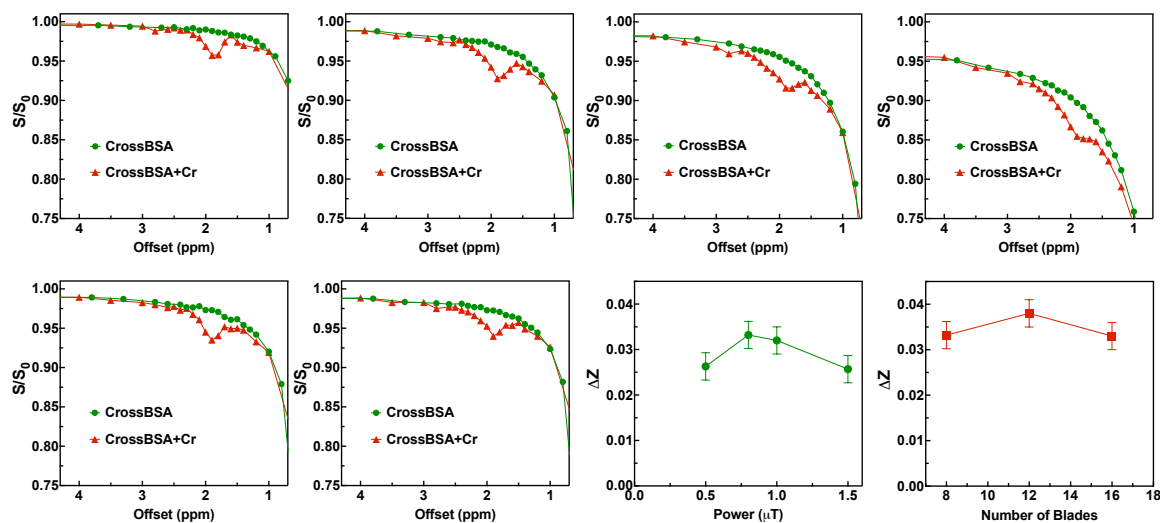


Figure 4: The Z-spectra between 1-5 ppm for the Cr+10%CrossBSA and 10%CrossBSA recorded with different powers and number of blades.

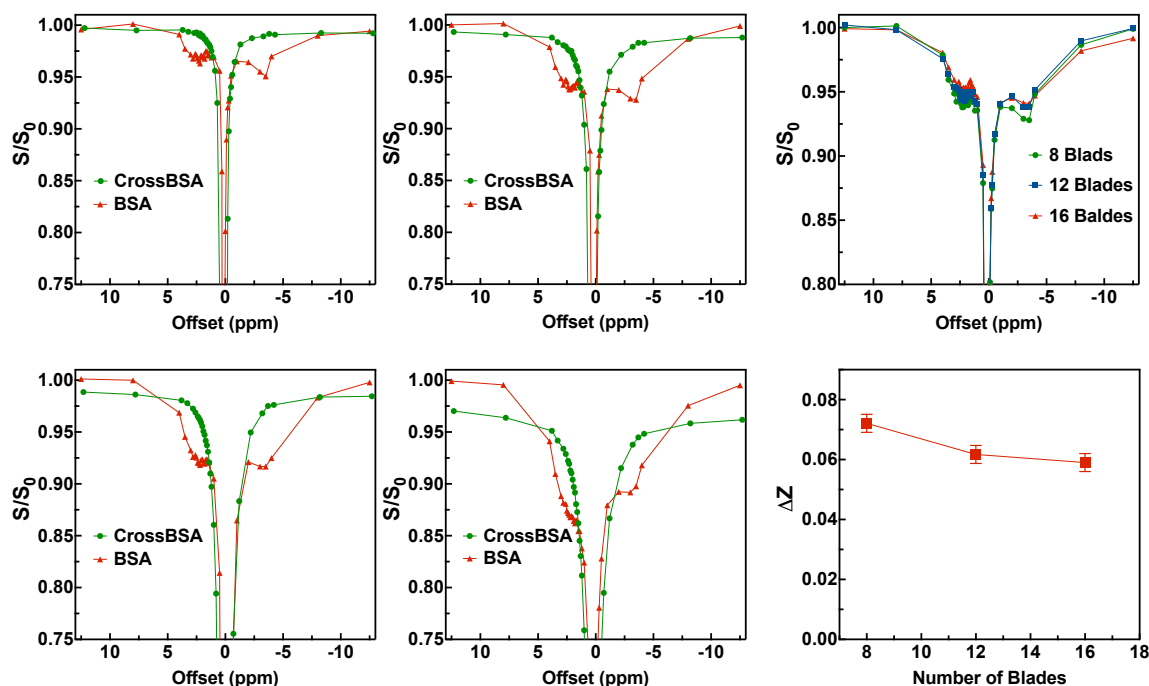


Figure 5: The full Z-spectra of the Cr+10%CrossBSA and 10%CrossBSA recorded with different powers and number of blades.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

NONE

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these

project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

NONE

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period, we will add new mice to the longitudinal scan to reach the number required for the significance to monitor the rNOE-CEST signal change with respect to the age up to 12 months. We will continue optimize the PROPELLER CEST method on human scanner by solving the B0/B1 inhomogeneity issues.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We developed one multi-slice ultra short echo time (UTE)-CEST sequence and established one automatic Matlab process pipeline for the multi-slice high-resolution rNOE images insensitive to motion. We demonstrated that the rNOE-CEST signal detected by CEST-UTE on AD mouse is associated with the A β accumulation due to the line broadening of amide and aliphatic proton signal in proteins introduced by protein aggregation. The current technique not only provide a new clinic-ready MRI technique that detect and evaluate the AD associated protein aggregation, but also can be used for detecting other metabolites in many tissues such as glycogen in liver.

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The current UTE-CEST method can be applied for the early diagnosis of the Alzheimer's disease (AD), and is also important for dynamically monitoring the effects of new therapeutic agents on the clearance of the aggregated proteins. The method may potentially be a marker for studying protein aggregation process in other diseases such as Huntington's disease and cirrhosis in the liver.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

The project may lead to an MRI diagnosis technology that can transfer to entities in medical imaging industry.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Upon the successful completion of this proposal, we anticipate developing a new clinic-ready MRI biomarker that detect and evaluate the AD associated protein aggregation. Such a non-invasive translational technique would play a pivotal role for definitive diagnosis of AD and for differentiating AD from other dementias. Early diagnosis of AD has significant impact on its management, and may allow early therapeutic interventions, such as amyloid immunization or inhibitors of beta-secretase (BACE). Furthermore, the new biomarker can be applied to dynamically monitor the effects of new therapeutic agents on the clearance of the aggregated proteins in the AD patients after traumatic brain injury (TBI). Therefore, the proposed research is in line with the long-term goal of the PRARP's mission that reducing the burden on affected individuals. The proposed MRI biomarker provides a cost-effective and simple approach that allows the researchers in the health science to perform routine tests on the individuals after traumatic brain injury (TBI). Considering MRI has been routinely performed on the patients with TBI, the new rNOE-CEST MRI experiments will provide abundant information about the protein aggregation associated with AD disease without additional diagnosis instruments.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Due to the much less animal generated, I would like to reduce the effort of Dr Tong Li and Philips Wong, who are supposed to perform the pathology analysis and breeding animals, by 50% in the last year.

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

None

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We found it is challenging to generate the required number of mice at the similar time period. Hence, we will perform our longitudinal study by using several cohorts of mouse. The cohort of mouse will be separated by 3-4 months depend on the breeding the mouse.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

*Due to the COVID19 pandemic, some of the animals were euthanized as requested by the university. It will delay our study with new cohort of mice.
The postdoc working on this project also left the institute which further delayed our progress. Hence, we are focusing on publishing the results we obtain previously.*

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Chen, L., Wei, Z., Chan, K.W.Y., Cai, S., Liu, G., Lu, H., Wong, P.C., van Zijl, P.C.M., Li, T.*, **Xu, J.***, 2018. **Protein aggregation linked to Alzheimer's disease revealed by saturation transfer MRI**. *Neuroimage* 188, 380-390. Published, *acknowledgement of federal support* : yes.
2. Lin Chen, Zhiliang Wei, Kannie Chan, Yuguo Li, Kapil Suchal, Sheng Bi, Jianpan Huang, Xiang Xu, Philip Wong, Hanzhang Lu, Peter van Zijl, Tong Li*, Jiadi Xu* **“D-Glucose uptake and clearance in the tauopathy Alzheimer’s disease mouse brain detected by on-resonance variable delay multiple pulse MRI”**, *Journal of Cerebral Blood Flow & Metabolism*, (2020) <https://doi.org/10.1177/0271678X20941264>. Published, *acknowledgement of federal support* : yes.
3. Lin Chen*, Suyi Cao, Raymond C. Koehler, Peter CM van Zijl, Jiadi Xu, **“High-sensitivity CEST Mapping Using the Spatiotemporal Correlation Enhanced Method”**, *Magnetic Resonance in Medicine*, (2020) <https://doi.org/10.1002/mrm.28380>. Published, *acknowledgement of federal support* : yes.
4. Lin Chen, Michael Schär, Kannie WY Chan, Jianpan Huang, Zhiliang Wei, Hanzhang Lu, Qin Qin, Robert G Weiss, Peter CM van Zijl, Jiadi Xu*, **“In vivo imaging of phosphocreatine with artificial neural networks”**, *Nature Communications*, 11, 1072 (2020). Published, *acknowledgement of federal support* : yes.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a*

periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

NONE

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Chen L, Barker P, Weiss R, van Zijl P, Xu J, "Creatine and phosphocreatine mapping of mouse skeletal muscle by a polynomial and Lorentzian line-shape fitting CEST method" Proceedings 27th ISMRM, 1345, Montreal, Canada, May 2019.
2. Chen L, Schär M, Chan K, Huang J, Wei Z, Lu H, Qin Q, Weiss R, van Zijl P, Xu J, "High-resolution phosphocreatine mapping of human skeletal muscle by artificial neural network-based chemical exchange saturation transfer MRI at 3T", Proceedings 27th ISMRM, 0048, Montreal, Canada, May 2019.
3. Chen L, Wei Z, Chan K, Huang J, Xu X, Wong P, Lu H, van Zijl P, Li T, Xu, J "Functioning of the glucose transporter and glymphatic systems in the tauopathy AD mouse brain studied by onVDMP MRI and D-glucose infusion" Proceedings 28th ISMRM, 1935, Virtual Meeting 2020.
4. Chen L, Schär M, Chan K, Huang J, Wei Z, Lu H, Qin Q, Weiss R, van Zijl P, Xu J, "High-resolution phosphocreatine mapping using artificial neural network-based CEST MRI at 3T: A validation study" Proceedings 28th ISMRM, 0343, Virtual Meeting 2020.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

<https://pages.jh.edu/~jxu37/resource.html>

My lab website provide links for disseminating the methods.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

UTE-CEST method

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life.

UTE-CEST sequence and the corresponding imaging processing software.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

*Name: Jiadi Xu
Project Role: PI
Nearest person month worked: 2
Contribution to Project: Dr. Xu performed MRI experiment, pulse programing, and oversee the progress of the whole project including writing the papers and progress report.*

*Name: Peter van Zijl
Project Role: co-investigator
Nearest person month worked: 1
Contribution to Project: Dr. van Zijl will provide support for the statistical analysis of the MRI images and interpretation of the MRI contrast.*

*Name: Lin Chen
Project Role: Postdoc
Nearest person month worked: 2
Contribution to Project: Dr. Lin will process the images and the statistical analysis.*

*Name: Tong Li
Project Role: co-investigator
Nearest person month worked: 2
Contribution to Project: The crossbred of mouse and following DNA sequencing will be operated by Dr. Li. The histological analysis will be complete by D. Li.*

*Name: Philips Wong
Project Role: co-investigator
Nearest person month worked: 1
Contribution to Project: Dr. Wong will provide support for the pathological process and interpretation of the images.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A*

duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

- 9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text.*

D-Glucose uptake and clearance in the tauopathy Alzheimer's disease mouse brain detected by on-resonance variable delay multiple pulse MRI

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Abstract

In this study, we applied on-resonance variable delay multiple pulse (onVDMP) MRI to study D-glucose uptake in a mouse model of Alzheimer's disease (AD) tauopathy and demonstrated its feasibility in discriminating AD mice from wild-type mice. The D-glucose uptake in the cortex of AD mice ($1.70 \pm 1.33\%$) was significantly reduced compared to that of wild-type mice ($5.42 \pm 0.70\%$, $p = 0.0051$). Also, a slower D-glucose uptake rate was found in the cerebrospinal fluid (CSF) of AD mice ($0.08 \pm 0.01 \text{ min}^{-1}$) compared to their wild-type counterpart ($0.56 \pm 0.1 \text{ min}^{-1}$, $p < 0.001$), which suggests the presence of an impaired glucose transporter on both blood–brain and blood–CSF barriers of these AD mice. Clearance of D-glucose was observed in the CSF of wild-type mice but not AD mice, which suggests dysfunction of the glymphatic system in the AD mice. The results in this study indicate that onVDMP MRI could be a cost-effective and widely available method for simultaneously evaluating glucose transporter and glymphatic function of AD. This study also suggests that tau protein affects the D-glucose uptake and glymphatic impairment in AD at a time point preceding neurofibrillary tangle pathology.

Keywords

Glucose uptake, Alzheimer's diseases, on-resonance variable delay multiple pulse, dynamic glucose enhanced, chemical exchange saturation transfer, tau pathology

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Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases and its prevalence increases with age.¹ Early diagnosis of AD is needed for several reasons, especially possible timely pharmacological intervention to slow the rate of disease progression.² In addition to its two main pathological hallmarks,

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i.e. the amyloid-beta ($A\beta$) plaques and neurofibrillary tangles, a diminished number of glucose transporters at the blood–brain barrier (BBB) is another trait of AD.³ Reduced glucose uptake in hippocampus and cortical regions have been observed in individuals who are at genetic risk for AD, or have mild or no cognitive impairment but continue developing to AD.⁴ The glucose-uptake impairment precedes brain atrophy and neuronal dysfunction, and therefore can provide valuable information for early diagnosis of AD.⁵ In addition to the BBB pathway, there is an alternative pathway to circulate glucose in the brain, the glymphatic system (Figure 1(a)). This is a paravascular route that facilitates cerebrospinal fluid (CSF) flow through the brain parenchyma.⁶ After entering the CSF through the blood–cerebrospinal fluid barrier (BCSFB), glucose can be recirculated to the interstitial fluid (ISF) in parenchyma through the glymphatic pathway.⁷ Therefore, characterization of the glucose uptake in CSF may add a new dimension to evaluate the functions of the BCSFB and the glymphatic system. Currently, the examination of CSF–ISF exchanging processes by MRI has been achieved mainly by intracranial⁸ or intrathecal infusion⁹ of gadolinium-based contrast agents. Although intrathecal infusion has been applied to human studies, it is still a highly

invasive method and might not be ideal for routine and repeated measurements on patients. Also, the safety of gadolinium-based agents is currently a concern as they may be retained in the deep gray matter nuclei for longer periods of time.¹⁰ In the current study, we also aim to explore the possibility of assessing the glymphatic pathway using D-glucose as a natural biodegradable tracer.

For several decades, glucose uptake and metabolism in brain have been assessed non-invasively using 2-[¹⁸F] fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET).¹¹ Despite its great detection sensitivity, the high-cost of FDG-PET hampers its wide clinical application. In addition, the use of a radioactive tracer is not ideal for repeated measurements, especially when combined with CT for anatomical referencing.¹² Alternative ways to measure glucose uptake and utilization are ¹³C and ¹H in vivo magnetic resonance spectroscopy (MRS),^{13,14} as well as a recent deuterium metabolic imaging method.¹⁵ Although these MRS approaches have shown great success in studying fundamental metabolic processes during glucose infusion, they are difficult to implement in the clinical environment due to the low detection sensitivity and limited availability of ¹³C and ²H coils and related transmit/receive systems.

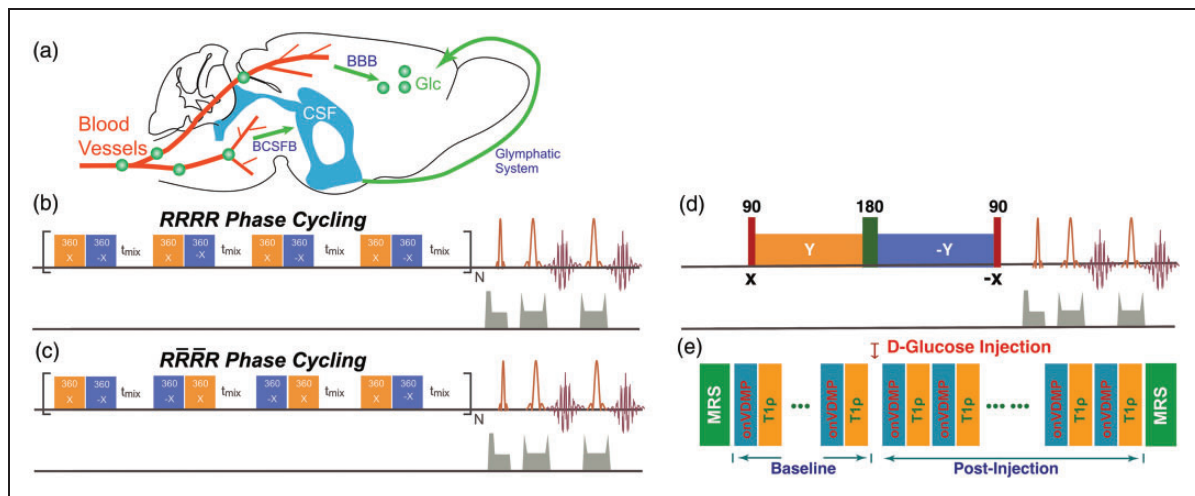


Figure 1. (a) Illustration of the glucose transport pathways in mouse brain after intravenous infusion of glucose. Glucose in blood crosses the BBB's luminal and abluminal membranes and reaches parenchyma. Part of the glucose rapidly enters the CSF through the BCSFB and recirculates to the parenchyma through the glymphatic system. The onVDMP saturation module with (b) RRRR and (c) RRRR phase cycling is composed of a train of binomial pulses separated by a delay t_{mix} . The flip angle of the binomial pulse is 360°. In the current study, a simple binomial pulse composed of two high power pulses with opposite alternating phase ($p\bar{p}$) was used. (d) $T_{1\rho}$ method: the water magnetization is first flipped by a hard 90° pulse and then locked by a spin-lock pulse. The hard 90° pulse following the spin-lock pulse flips the magnetization back to the Z-axis for MRI readout. The 180° pulse in the middle of spin-lock pulse was used to improve the robustness to the B_0 inhomogeneity. (e) Diagram for the DGE study using both onVDMP and $T_{1\rho}$ sequences, which were collected in an interleaved manner. Two MRS spectra were collected before and after the experiment to confirm the glucose and other metabolites variation during glucose infusion in brain. BBB: blood–brain barrier; CSF: cerebrospinal fluid; BCSFB: blood–cerebrospinal fluid barrier; MRS: magnetic resonance spectroscopy; onVDMP: on-resonance variable delay multiple pulse.

Glucose chemical exchange saturation transfer (glucoCEST) MRI is a recently developed technique^{16,17} that can detect unlabeled glucose at physiologically relevant concentrations using proton-only MRI scanners without the requirement of additional hardware. Among various glucoCEST methods, on-resonance variable delay multiple pulse (onVDMP) MRI is promising due to its excellent labeling efficiency and sensitivity.^{18–20} In this study, we applied onVDMP MRI to detect cerebral D-glucose uptake and CSF clearance in AD mice and demonstrate its feasibility in discriminating AD from normal mice.

Methods

onVDMP sequence with phase cycling scheme

The conventional onVDMP sequence is composed of a train of binomial pulses followed by an image acquisition module, as shown in Figure 1(b).^{18–20} As a previous VDMP study suggested,²¹ the fast-exchanging signal component decays during the mixing time. Hence, the mixing time between binomial pulses was set to zero in this study of hydroxyl groups in D-glucose under physiological conditions and the offset of the binomial pulses was set to the water resonance. In practice, however, a water resonance shift may be induced by glucose infusion,²² which may affect the onVDMP MRI signal. Also, pulse imperfections caused by B_1 inhomogeneity may cause image artifacts. These issues can be well addressed by phase cycling, a technique that has been commonly used in NMR composited pulses to cancel out the undesirable effects from B_0 and/or B_1 inhomogeneities.²³ Here, we introduced a new phase cycling scheme to onVDMP sequence to improve its robustness against water resonance shift and B_1 inhomogeneity, as indicated in Figure 1(c). The phase cycling scheme is $R\bar{R}\bar{R}R$, where R and $\bar{R}$ refer to the phase and inverted phase, respectively. The efficiency of phase cycling was first demonstrated by Bloch simulation and then validated with *in vivo* experiments, as shown in online Supplementary Materials Section S1.

Animal preparation

All animal experiments were performed under the protocols based on the NIH Guidelines for Care and Use of Laboratory Animals. All experiments were performed in accordance with the Animal Research Reporting In Vivo Experiments guidelines,²⁴ and approved by the Johns Hopkins University animal care and use committee. Four female Tau4RAK (Tau) mice²⁵ with an age of 7–8 months and four female age-matched C57BL/6J mice of comparable bodyweight (24 ± 2 g) were used. Two age-matched BALB/c mice were used for the dynamic

monitoring of D-glucose uptake using MRS. Three female age-matched C57BL/6J mice were used for a control experiment with saline infusion. Comparisons between the onVDMP and $T_{1\rho}$ method, together with MRS measurement before and after D-glucose infusion, were carried out on three female age-matched C57BL/6J mice. The Tau mice were bred at Johns Hopkins University and the onset of tau tangles starts at the age of six months.²⁵ Many studies,^{26–28} including several failed anti-amyloid agent clinical trials,²⁹ have suggested that neurofibrillary tangles instead of plaques drive the cognitive decline and atrophy of the brain in AD patients. The new Tau mouse model (Tau4RAK) expresses a human tau repeat domain that is capable of inducing the pathological conversion of wild-type (WT) mouse tau and exhibits the age and neuritic plaque dependent tau pathology seen in AD patients.²⁵ This model provides us with a unique opportunity to demonstrate the use of the onVDMP MRI approach for assessing changes in brain glucose uptake and CSF clearance through the glymphatic pathway in early AD.

MRI experiments

All MRI experiments were performed on a horizontal bore 11.7 T Bruker Biospec system (Bruker, Ettlingen, Germany) equipped with actively shielded gradients with a maximum strength of 74 Gauss/cm. A 72 mm quadrature volume resonator was used as a transmitter and a 2×2 mouse phased array coil for acquisition. All animals were anesthetized using 2% isoflurane in medical air, followed by 1–1.5% isoflurane for maintenance during the MRI scan. The mouse head was positioned using a bite bar and two ear pins. During the MRI scan, mice were placed on a water-heated animal bed. The tail vein was cannulated with a catheter for D-glucose infusion before MRI scans. Respiratory rate was monitored via a pressure sensor (SAII, Stony Brook, NY, USA) and maintained at 40–60 breaths per minute. The B_0 field over the mouse brain was adjusted using field-mapping and second-order shimming.

According to a previous study,²⁰ the optimal signal-to-noise ratio of onVDMP signal can be achieved by setting the length of saturation module comparable to the T_2 value of tissue of interest. To obtain satisfactory onVDMP signal for CSF, the T_2 value of CSF was measured by Car–Purcell–Meiboom–Gill preparation module³⁰ with 10 echo times ranging from 20 ms to 340 ms and rapid acquisition with refocused echoes (RARE) imaging module with an echo time (TE) of 120 ms, TR = 10 s, RARE factor = 23, slice thickness = 1 mm, a matrix size of 196×128 , and a field-of-view (FOV) of 20×10 mm².

To validate that $R_{1\rho}$ theory can be applied for the analysis of onVDMP data, we compared the dynamic

glucose enhanced (DGE) signals obtained by onVDMP MRI and a classical on-resonance spin-lock ($T_{1\rho}$) method.^{31–36} In the onVDMP DGE measurements of the brain parenchyma, 12 binomial pulses with 36 ms length were implemented. The duration of each binomial pulse pair was 3 ms. The $T_{1\rho}$ sequence is illustrated in Figure 1(d). Two Gaussian 90° pulses (1 ms width) were used for the preparation and flip-back pulses and one Mao pulse³⁷ (5 ms width) was applied for the 180° refocusing pulse. The total length of the $T_{1\rho}$ module was set to 36 ms, which was identical to the onVDMP length. The onVDMP and $T_{1\rho}$ DGE curves were collected in an interleaved manner on mouse brain as illustrated in Figure 1(e). Two MRS spectra were collected before and after the DGE experiments to validate the glucose concentration change after D-glucose infusion. The in vivo MRS data were acquired on a voxel of $3 \times 3 \times 3 \text{ mm}^3$ in the thalamus using a STEAM sequence (echo time (TE) = 3 ms, mixing time (TM) = 10 ms, repetition time (TR) = 2.5 s, average number (NA) = 128) with outer volume suppression. First- and second-order shims were adjusted for each VOI using a field-mapping method. The water signal was suppressed by the variable power radiofrequency pulses and optimized relaxation delays (VAPOR) method.³⁸ The image slice was selected to also enable us to observe the superior sagittal sinus (SSS), voxels in which were used to extract the D-glucose concentration in the blood vessels. Prior to the experiments, one Time-Of-Flight MRA experiment covering the whole mouse brain was collected to locate the SSS. Other imaging parameters were: TR/TE = 2.5 s/5 ms, RARE factor = 23, slice thickness = 1 mm, a matrix size of 196×128 within a FOV of $20 \times 10 \text{ mm}^2$. The scan time for each onVDMP experiment was fixed to 7.5 s and DGE images were acquired continuously for 35 min. A bolus of 0.15 mL 50% w/w D-glucose (0.5 g/mL, clinical-grade dextrose, Hospira, Lake Forest, IL) was given at 10th min of the dynamic experiment through the tail vein using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The first five minutes pre-scan were used as dummy scans to make sure the mouse was stabilized in the scanner.

The length of the saturation module of an onVDMP sequence is proportional to the number of binomial pulses. For the current study, two onVDMP preparations (12 and 100 pulses), i.e. of different saturation-module lengths (i.e. 36 ms and 300 ms), were repeated in an interleaved fashion. A 10 min pre-scan was performed before glucose infusion and the first five minutes pre-scans were discarded. DGE images were acquired continuously through 27.5 min post-infusion. The MRI readout and glucose infusion protocol were identical to the one used in the above experiments. Osmolarity control experiments were performed on

three C57BL/6J mice by injecting 3% saline solution (0.15 mL in one minute). Since the osmolarity of 3% saline is about 2.5 times lower compared to the 50% glucose, we evaluate the response in proportion. The experiments revealed the stability of the onVDMP MRI signal, as well as the potential non-glucose contributions to the DGE signal, e.g. due to osmolarity differences between blood and the infusate.

Dynamic D-glucose uptake detected by MRS

Dynamic in vivo MRS spectroscopy was performed to validate the change of D-glucose concentration in brain tissue after D-glucose infusion. An infusion protocol identical to that used in the DGE MRI experiments was adopted. The MRS experiments were identical to the one used in the onVDMP and $T_{1\rho}$ DGE comparison experiment except for NA = 64, which leads to a scan time of 2.6 min for each MRS.

Data analysis

The onVDMP and on-resonance spin-lock MRI approaches closely resemble each other and it has been shown that CEST approaches such as onVDMP MRI can be also described using $T_{1\rho}$ theory.^{39,40} The water signal intensity (S) after applying onVDMP sequence can be expressed as

$$S = S_0 e^{-T_{\text{onVDMP}} R_{1\rho}} \quad R_{1\rho} = R_2 + R_{ST} \quad (1)$$

where S_0 is the water signal intensity without saturation, R_2 is the transverse relaxation rate of water without additional solution components, and R_{ST} accounts for the contributions from saturation transfer components from all exchangeable protons. With glucose infusion, the change of $R_{1\rho}$ is given by

$$\Delta R_{1\rho} = \Delta R_2 + R_{glc} \quad (2)$$

where ΔR_2 is the change of transverse relaxation rate of water induced by the exchange between the hydroxyl protons from glucose and water^{41,42} and R_{glc} is the change in rate of saturation transfer components due to glucose infusion. Because $\Delta R_{1\rho}$ induced by glucose infusion is relatively small,³² the normalized change of water signal intensity ΔZ can be approximated as (online Supplementary Materials Section 2)

$$\begin{aligned} \Delta Z &= (S_{\text{base}} - S) / S_{\text{base}} \\ &= 1 - e^{-T_{\text{onVDMP}} \Delta R_{1\rho}} \approx T_{\text{onVDMP}} \Delta R_{1\rho} \end{aligned} \quad (3)$$

where S_{base} is the referenced water signal intensity. In this study, the average of water signal intensity before

glucose infusion was used as S_{base} . The area-under-curve (AUC) image, which reflects glucose uptake and retention, was calculated by averaging N images within a certain time period

$$AUC = \sum_{n=1}^N \Delta Z(n) / N \quad (4)$$

To correct for motion between DGE images, image registration was done using Medical Imaging Registration Toolbox.⁴³ The regions of interest (ROIs) were selected by hand with the reference of mouse brain atlas (<https://mouse.brain-map.org>).

The total creatine (tCr) concentration was measured using LCModel⁴⁴ from the in vivo MRS and used as a concentration reference for D-glucose. The glucose uptake ($[\Delta Glc]$) after glucose administration was determined using the area ratio between the change in the D-glucose peaks (3.4 and 4.0 ppm) in the difference spectrum and the tCr methyl peak (3 ppm) in the spectrum ($R_{Glc/tCr}$) with the known tCr concentration ($[tCr]$)^{45–47} following

$$[\Delta Glc] = (3/5) \cdot R_{Glc/tCr} [tCr] \quad (5)$$

where 3/5 is the proton ratio between the tCr methyl NMR peak (three protons) and the D-glucose NMR peaks between 3.4 ppm and 4.0 ppm (five protons; note that we exclude the H2 proton that falls outside this range). The peak areas were extracted by fitting the tCr peak at 3 ppm as well as the two composite D-glucose peaks using Lorentzian functions, as illustrated in online Supplementary Figure S3.

The glucose uptake curves measured by ¹H MRS and DGE MRI were fitted using exponential functions to allow quantitative comparison

$$\Delta DGE(t) = A_{DGE}(1 - e^{-\mu_{in,DGE} \cdot t}) \quad (6)$$

$$[\Delta Glc](t) = A_{MRS}(1 - e^{-\mu_{in,MRS} \cdot t}) \quad (7)$$

where $\Delta DGE(t)$ is the DGE signal difference determined by onVDMP MRI, and $[\Delta Glc](t)$ is the glucose concentration difference due to infusion determined by MRS. $\mu_{in,DGE}$ and $\mu_{in,MRS}$ are the glucose uptake rates determined by onVDMP DGE and MRS, respectively. A_{DGE} and A_{MRS} represent the amplitudes of glucose uptake at equilibrium measured by onVDMP and MRS, respectively. A two-sample t-test was performed on the fitting parameters between the AD and WT groups, and was considered statistically significant for $P < 0.05$ and highly significant when $P < 0.001$.

Sensitivity and specificity determination

The sensitivity and specificity in distinguishing AD mice from healthy mice was calculated using Receiver Operating Characteristic (ROC) plots on the extracted glucose uptake amplitudes (A_{DGE}) and rates ($\mu_{in,DGE}$) in parenchyma and CSF. The ROC curves were calculated by one Matlab function `roc_curve` (www.mathworks.com/matlabcentral/fileexchange/52442-roc-curve).

Results

Measurement of T_2 value for CSF

Representative images of mouse brain as a function of echo time are shown in Figure 2(a). From the images, the signal of brain parenchyma attenuates much faster compared to that of CSF. This can also be observed from the signal intensities of cortex and CSF as a function of echo time, as shown in Figure 2(b). With an echo time of 120 ms, the cortical signal intensity is only 8.4% with respect to its maximum value (at $TE=0$ ms), while CSF signal intensity still maintains 74.4%. By fitting the signal intensities, the T_2 values of cortex and CSF were determined to be 44 ± 5 ms and 380 ± 25 ms, respectively. According to our previous report, the optimal signal-to-noise for the onVDMP method is obtained when the length of the saturation module is comparable to the T_2 value of interested region.²⁰ Therefore, onVDMP sequences with 12 binomial pulse pairs (36 ms) and 100 binomial pulse pairs (300 ms) were used to detect the glucose uptake in tissue and CSF, respectively.

Comparison of DGE signal obtained by onVDMP and $T_{1\rho}$

Typical onVDMP and $T_{1\rho}$ images are plotted in Figure 3(a) and (b), respectively, and show similar contrasts. The DGE curves for the parenchyma of WT mice measured by onVDMP and $T_{1\rho}$ MRI are presented in Figure 3(c). The two methods show similar curves with comparable A_{DGE} values of $3.81 \pm 1.30\%$ (onVDMP) and $3.63 \pm 1.92\%$ ($T_{1\rho}$), illustrating the equivalence of these two approaches.

Detection of blood DGE signal

The dynamic blood DGE signal in blood was assessed from the SSS onVDMP signal (Figure 4(a) and (b)). The blood DGE signal after D-glucose infusion ($n=3$) is plotted in Figure 4(c). From the result, the blood DGE signal increased rapidly during the one minute D-glucose infusion to around 6% of the water signal and stayed at the same concentration during the whole 30 min of experimental time. This result is

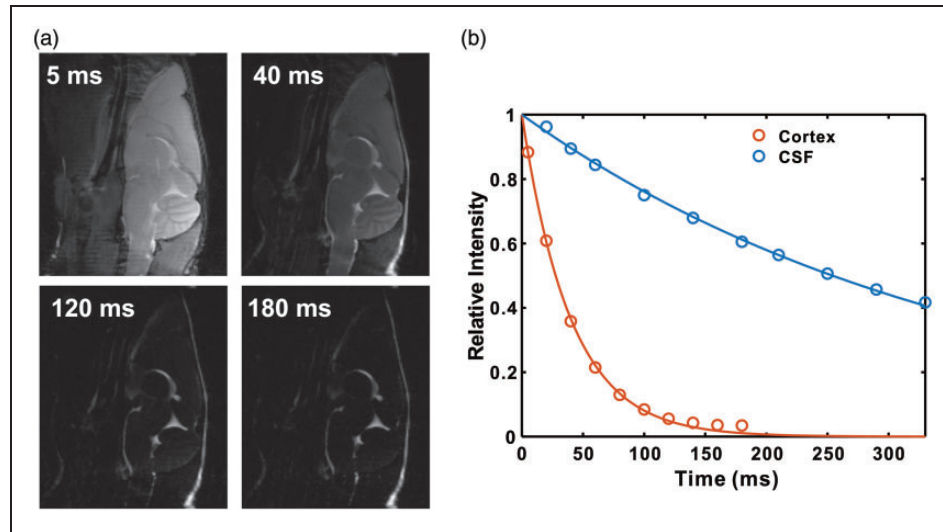


Figure 2. The T_2 values of cortex and CSF were measured using a CPMG preparation module followed by a RARE imaging module. (a) Representative sagittal images of mouse brain recorded with different echo times. (b) The signal decay as a function of echo time for tissue and CSF, respectively. CSF: cerebrospinal fluid.

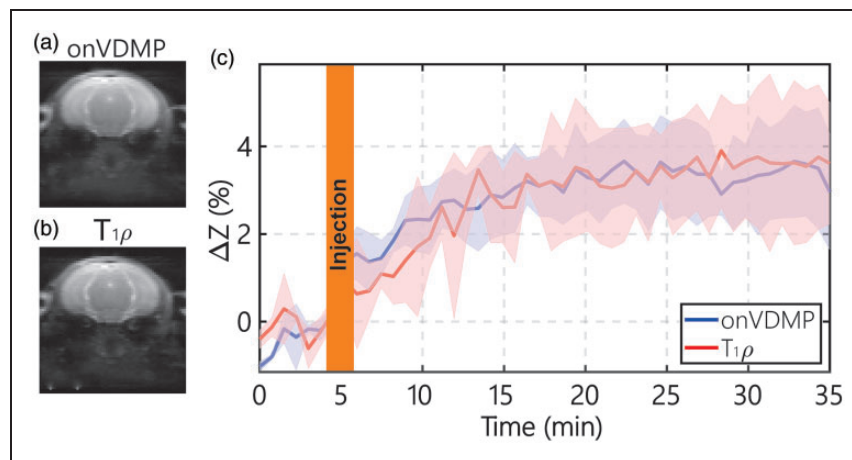


Figure 3. Comparison of the DGE curves during D-glucose infusion obtained with onVDMP and $T_{1\rho}$ sequences: typical onVDMP (a) and $T_{1\rho}$ (b) images are plotted to show the location of the slice and the image quality. (c) DGE curves of mouse parenchyma acquired by onVDMP MRI (blue) with 12 binomial pulse pairs (36 ms) and $T_{1\rho}$ imaging (red) with a saturation time of 36 ms on WT mice ($n = 3$; standard deviation indicated in light blue and red). The unit of ΔZ is %. onVDMP: on-resonance variable delay multiple pulse.

consistent with our recent study, in which the time dependence of the blood glucose concentration was confirmed outside the scanner.⁴⁸

D-Glucose uptake and clearance in the tauopathy AD mice

When studying the onVDMP images with 12 binomial pulse pairs (Figure 5(a) and (c)), the Tau mice showed an obvious reduction in brain volume compared to WT mice. This was further validated with a brain volume

measurement. The volume was calculated by delineating the whole brain boundary slice-by-slice on high-resolution multi-slice T2-weighted images with the reference of mouse brain atlas (<https://atlas.brain-map.org/>). The number of voxels inside the brain was multiplied with the voxel volume to yield the total brain volume in mL. The brain volumes were 0.53 ± 0.01 mL for WT mice and 0.45 ± 0.02 mL for Tau mice, which is significantly different ($p = 0.003$, $n = 4$). The CSF volume of Tau AD mice ($11.8 \pm 1.8 \mu\text{l}$) trended to be slightly higher than that of WT

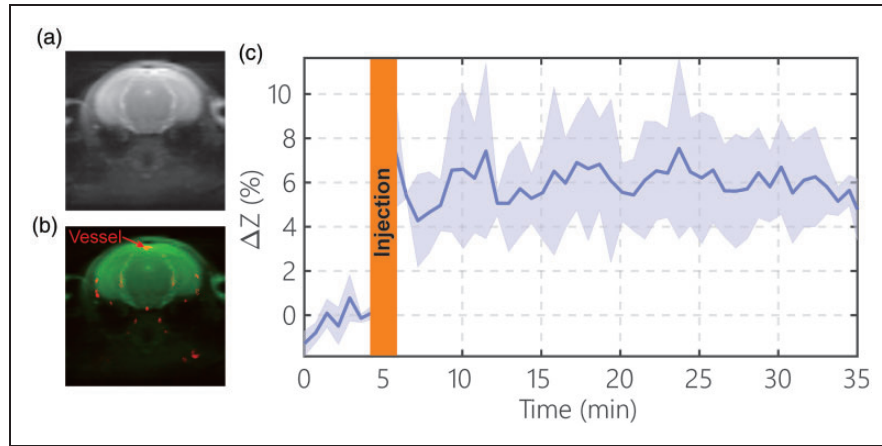


Figure 4. The blood D-glucose level after D-glucose infusion (one minute, 150 μ L, 50% dextrose) determined by the onVDMP method. A typical onVDMP image (a) and overlaid blood vessel map (red) (b) determined by TOF MRI show the location of the superior sagittal sinus (SSS), which is used for measuring the blood D-glucose signal changes. (c) DGE curve from the SSS acquired using an onVDMP sequence with 12 binomial pulse pairs (36 ms) ($n = 3$; standard deviation indicated in light blue). The unit of ΔZ is %.

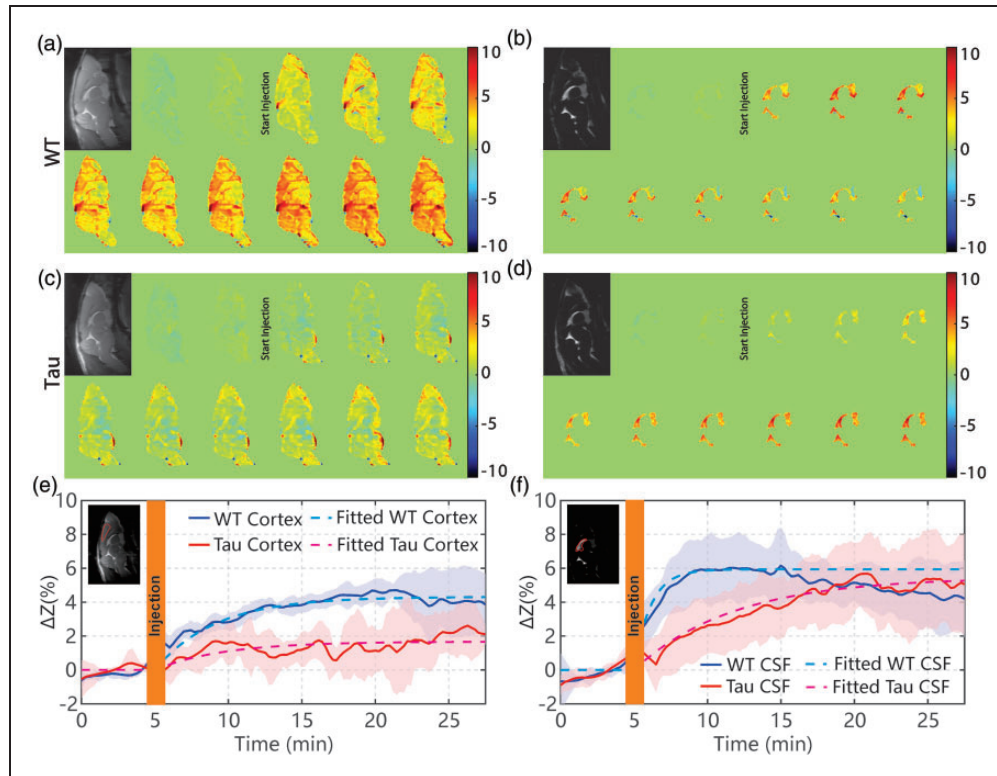


Figure 5. Dynamic AUC maps for WT ((a) and (b)) and Tau ((c) and (d)) mice over the 27.5 min DGE experiment. The brain tissue maps were recorded using the onVDMP sequence with 12 binomial pulse pairs ((a) and (c)), while the CSF maps were obtained with 100 binomial pulse pairs ((b) and (d)). Each dynamic AUC map was calculated by averaging 10 successive DGE images, which lead to a time window of 150 s. The averaged time-resolved DGE curves for the (e) cortex and (f) CSF of WT and Tau mice. The ROIs used for determining the DGE curves are indicated in the inserted images with red lines. Shaded regions in the graphs represent the standard deviation of the curves ($n = 4$). The averaged DGE curves (solid lines) and the corresponding curves from fitting with equation (6) (dotted lines) are shown. The unit of ΔZ is %.

WT: wild-type; Tau: Tau4R Δ K; CSF: cerebrospinal fluid.

mice, but this difference was not significant ($9.2 \pm 1.4 \mu\text{L}$; $p=0.11$, $n=4$). We also measured the body weight of WT (24.33 ± 0.47 g, $n=4$) and AD (23.75 ± 2.38 g, $n=4$) mice, and no significant difference was observed ($p=0.739$). Dynamic AUC maps were utilized to monitor the D-glucose uptake in mouse brain and CSF for both Tau and WT mice (Figure 5(a) to (d)). Each dynamic AUC map was calculated by averaging 10 successive DGE images, which corresponds to a time-interval of 150 s. The averaged DGE curves of both WT ($n=4$) and Tau ($n=4$) mice for the cortex are plotted in Figure 5(e) and (f), respectively. The dynamic maps and curves show that the D-glucose uptake is slow in the parenchymal ROI (cortex) of both WT and Tau mice, reaching a plateau after about 20 min. The fitted A_{DGE} of WT parenchyma ($5.42 \pm 0.70\%$) was significantly larger than that of Tau parenchyma ($1.70 \pm 1.33\%$, $p=0.005$). Both sensitivity and specificity were 100% for the A_{DGE} as seen from its ROC curve (online Supplementary section S4). The D-glucose uptake rate ($\mu_{in,DGE}$) was $0.15 \pm 0.03 \text{ min}^{-1}$ for WT mice and not significantly different from Tau mice ($0.13 \pm 0.01 \text{ min}^{-1}$, $p=0.511$). The DGE signal of CSF in WT mice built up quickly after D-glucose infusion and plateaued around 10 min, with an uptake rate of $0.56 \pm 0.1 \text{ min}^{-1}$. Interestingly, after

reaching a plateau, the DGE signal of WT CSF began to decay, as can be seen in both dynamic AUC maps and DGE curves. On the other hand, the DGE signal of CSF in Tau mice kept increasing after D-glucose infusion and reached a plateau only around 25 min with an uptake rate of $0.08 \pm 0.01 \text{ min}^{-1}$, which is significantly slower than WT ($p<0.001$). The CSF A_{DGE} values were similar between WT mice ($A_{DGE}=7.03 \pm 2.12\%$) and Tau mice ($A_{DGE}=6.72 \pm 2.22\%$, $p=0.871$). While the sensitivity and specificity were low for the CSF A_{DGE} , they were 100% for the CSF $\mu_{in,DGE}$ as seen from their ROC curves (online Supplementary section S4).

Saline infusion

The dynamic AUC maps, as well as the onVDMP curves from both brain parenchyma and CSF following the infusion of 3% saline water, are plotted in Figure 6. After saline infusion, small onVDMP signal changes were observed in brain parenchyma, about $0.9 \pm 1.1\%$ (Figure 6(c)). Note that the osmolarity of 3% is about 2.5 times lower compared to that of 50% glucose, which means the osmolarity effect of glucose will most likely be higher than $0.9 \pm 1.1\%$ to the parenchyma onVDMP signal. Different from the DGE signal introduced by D-glucose infusion where the DGE signal built up slowly, the onVDMP signal due to

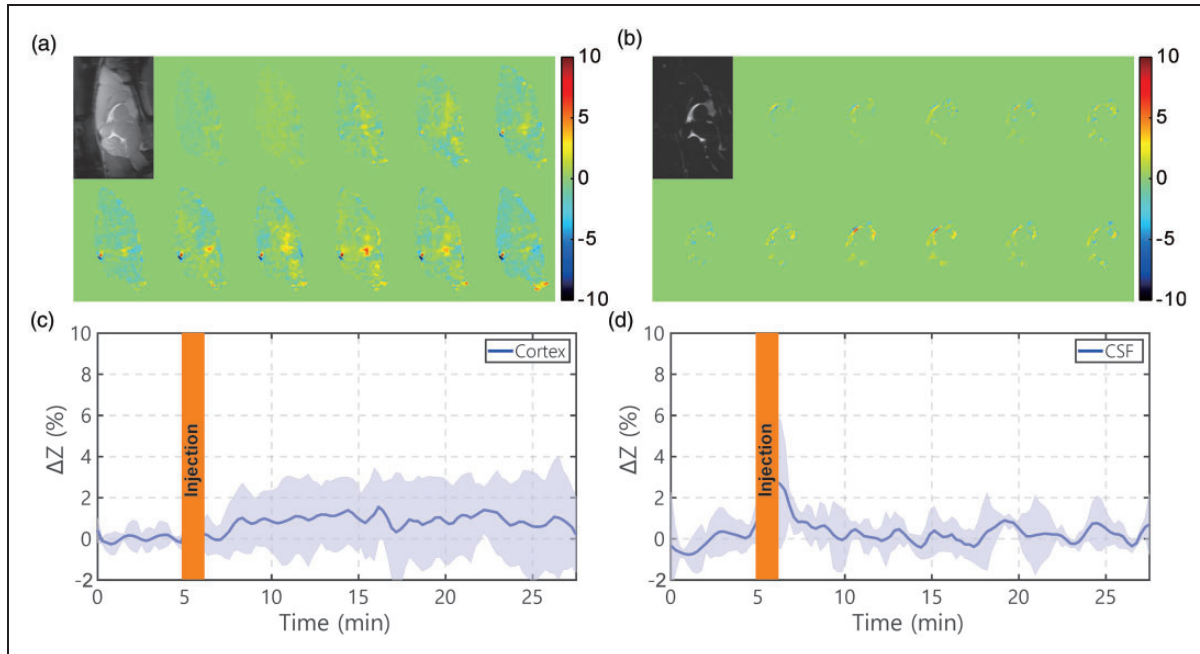


Figure 6. Dynamic AUC maps and DGE curves for WT mice infused with 3% saline (1 min, $150 \mu\text{L}$). The brain tissue AUC maps were recorded using the onVDMP sequence with 12 binomial pulse pairs (a), while the CSF AUC maps were obtained with 100 binomial pulse pairs (b). The infusion protocol and MRI experimental parameters were identical to those applied in Figure 4. ((c) and (d)) The averaged time-resolved DGE curves for (c) tissue and (d) CSF for WT mice ($n=3$; standard deviation indicated in light blue). The unit of ΔZ is %. CSF: cerebrospinal fluid.

saline infusion was close to constant during the period post-infusion. On the contrary, the onVDMP signal change due to saline infusion, while spiking during infusion, was negligible during the post-infusion period in the CSF.

MRS-based validation

Typical MRS spectra of the mouse brain before and after (30 min) D-glucose infusion, as well as the difference spectra for three typical time points (0 min, 5 min, and 30 min), are plotted in Figure 7(a). The two broad aliphatic D-glucose resonance peaks, around 3.4 ppm and 3.7 ppm, are increased substantially after D-glucose infusion as shown in the difference spectra. The signal-to-noise ratio (SNR) for the Cr peak in MRS was 34 ± 1.5 , while the Cramer-Rao lower bound (CRLB) for the fitted Cr peak at 30 min was $0.9 \pm 0.2\%$. The SNR for the D-glucose peaks in the difference spectra at 30 min was 2.0 ± 0.3 and CRLB for the fitted D-glucose peaks was $20 \pm 5\%$. The dynamic change of D-glucose concentration in Figure 6(b) showed a rapid buildup curve in the first 10 min. From the exponential fitting of the glucose uptake curves, A_{MRS} was determined to be 1.6 ± 0.3 mM and the D-glucose uptake rate $0.16 \pm 0.02 \text{ min}^{-1}$, which was comparable to the D-glucose uptake rate of WT mouse ($0.15 \pm 0.03 \text{ min}^{-1}$) obtained by the onVDMP method.

Discussion

In this study, we used phase-cycled onVDMP MRI to detect D-glucose uptake in mouse brain through the chemical exchange of protons between hydroxyl groups and water. The DGE curves obtained by onVDMP MRI on WT mice showed a similar pattern to those acquired on both human and animal brain using the $T_{1\rho}$ method,^{32,36} i.e. the DGE curves build up in the first 10–15 min after D-glucose infusion and then plateau or decay slowly. This was further confirmed by a comparison study between onVDMP and $T_{1\rho}$ methods on WT mice (Figure 3). The rate of D-glucose uptake obtained by the onVDMP method ($0.15 \pm 0.03 \text{ min}^{-1}$) was consistent with that measured by MRS ($0.16 \pm 0.02 \text{ min}^{-1}$) and previous ^{13}C MRS measurements ($0.136 \pm 0.011 \text{ min}^{-1}$),⁴⁹ which suggests that the change of onVDMP signal is correlated with the change in D-glucose concentration in brain. Besides onVDMP, there is an alternative CEST method, i.e. continuous-wave CEST (CW-CEST), to monitor D-glucose uptake in brain. However, due to a scale-down effect induced by magnetization transfer contrast from macromolecules,^{50,51} the signal sensitivity of CW-CEST is inferior to that obtained by onVDMP.²⁰ The DGE curve measured by CW-glucCEST on healthy mouse brain showed a bolus effect at the first three minutes and then dropped to small values,⁵² which is different from the DGE pattern determined by the onVDMP, $T_{1\rho}$ and MRS methods. Notice

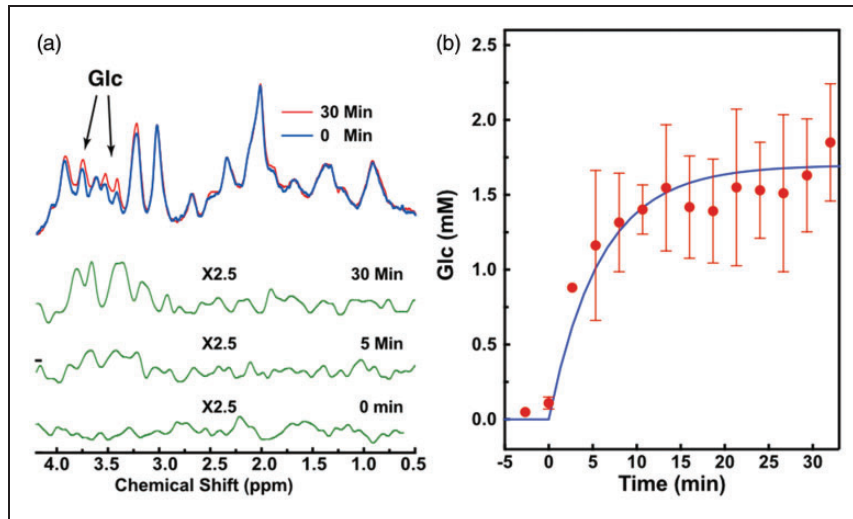


Figure 7. (a) In vivo MRS spectra for a typical mouse brain over a voxel in the thalamus before (blue) and after (red, 30 min) D-glucose infusion. Difference spectra at three time points (green) are also shown with two glucose peaks (3.4 ppm and 3.7 ppm) are indicated. (b) Dynamic MRS signal changes as a function of time relative to the start of D-glucose infusion ($n = 2$). The D-glucose to total creatine (tCr) ratios were calculated by integrating signal over the chemical shift range from 3.0 to 4.2 ppm in the difference spectrum and divided by the area of the tCr methyl group centered at 3 ppm. The solid line is the curve fitted with a single exponential function (equation (7)).

that glucose can also induce a R_2 relaxation change due to the chemical exchange between glucose hydroxyl protons and water,^{41,42} and this R_2 relaxation change also contributes to the change of onVDMP signal as indicated in equations (2) and (3), which is similar to the $T_{1\rho}$ -based methods. When detecting D-glucose in CSF, the scaling effect from MTC will be minimized. Furthermore, the R_2 of CSF is also much smaller than in parenchyma, which enabled us to detect D-glucose in CSF with much higher detection sensitivity at longer preparation times.

When assuming that the DGE signal arises solely from the $R_{1\rho}$ value variation introduced by D-glucose, a D-glucose concentration of 4.47 mM increment in CSF for WT mice can be estimated from a D-glucose phantom calibration (online Supplementary section S5). However, a much higher D-glucose concentration is estimated (26.5 mM) in brain parenchyma with 4% DGE signal (online Supplementary section S5). This value is far higher than the D-glucose concentrations measured by MRS method (1.5–1.7 mM) (Figure 7 and online Supplementary Figure S3). This indicates that the calibration from phantoms applies only to CSF, which makes sense as CSF more closely resembles a sugar solution phantom. The *in vivo* onVDMP signal in parenchyma detects all fast exchanging protons, and other metabolites (e.g. products of D-glucose metabolism) may also contribute to the change in the onVDMP signal. In addition, the background signal interference is more complicated, even though that should in principle not affect a rate determination. From the saline injection results, it is expected that the osmolarity effect of glucose also contributes to the parenchymal onVDMP signal. Although the exact contributions of the DGE signal in parenchyma are still not fully understood, the above studies suggest that the DGE signal is correlated with D-glucose concentration and can be used for monitoring the D-glucose uptake and clearance processes.

The D-glucose uptake in the brain is related to D-glucose delivery, transport, and metabolism. From the results shown in Figure 5, the D-glucose uptake in the cortex of Tau mice is significantly reduced compared to that of WT mice (signal intensities of $1.6 \pm 1.3\%$ vs $4.3 \pm 1.3\%$, $p = 0.016$). This is consistent with the observation that the D-glucose uptake is impaired in AD brain due to the reductions of both GLUT1 and GLUT3 expression at the BBB.³ Few studies have been performed to measure glucose uptake in brain CSF. In the current study, we employed an onVDMP sequence with a long saturation module to suppress the parenchymal signal, enhancing the detection sensitivity of CSF. Notice that such an approach would not work for MRS, as the T_2 of the D-glucose protons is short, but here we are studying the water signal in CSF. The

buildup process of the DGE curve in CSF reflects the D-glucose delivery and transport at the BCSFB. From the dynamic AUC maps and curves in Figure 5, Tau CSF has much slower D-glucose uptake compared to the WT CSF, which, assuming similar delivery, suggests that the D-glucose transporter at the BCSFB is impaired in AD brain. From previous studies, D-glucose can alternatively be redistributed to brain parenchyma through a CSF and ISF exchanging process, i.e. the glymphatic pathway,⁸ and the glymphatic pathway of AD brain has been reported to be dysfunctional.⁶ This may explain the observation that the DGE signal of WT CSF began to decay after reaching a maximum of around 15 min, while the DGE signal of Tau CSF kept increasing. As demonstrated in Figure 4, the blood DGE signal remained at a constant level after D-glucose infusion, which supports the standpoint that the glymphatic pathway plays a role in glucose transport into brain.⁵³ The evidence shown in this study suggests that D-glucose as a natural biodegradable contrast agent has the potential to be a non-invasive method to detect CSF–ISF exchange and reveal the function of glymphatic system. However, further validation is required.

Glucose uptake in amyloid precursor protein (APP) AD mouse models has been intensively studied with FDG-PET or *ex vivo* brain autoradiography methods⁵⁴ as well as our recent study using DGE MRI.⁴⁸ It was found that the glucose uptake in APP mouse brain is close to or even higher than the age-matched WT mice at the age of 5–10 months for several transgenic APP mouse models, such as 5XFAD,⁵⁵ Tg2576,⁵⁶ and APP/PS1.⁵⁴ However, the impact of tau protein alone on the glucose uptake in AD has not been well investigated. The current study provides one some initial evidence that the toxic tau protein instead of A β protein drives the glucose uptake impairment commonly seen in AD, which is consistent with the observation that glucose transporter decreases or hypometabolism are correlated to the density of neurofibrillary tangles but not A β in human brains.⁵⁷ Previous pathology studies on this animal model showed that the burden of tau tangles occurs by around 12 months, while the tangles begin to appear at the age of 6–9 months.²⁵ Thus, the glucose-uptake impairment appears to precede the AD tau tangle pathology.

The onVDMP technique can detect unlabeled sugars, which could offer considerable financial savings, and simplify the imaging procedure by removing the requirement for the production and delivery of [¹⁸F] FDG. Further improvements of the onVDMP experiment are still possible. In this study, we set the baseline scan time to 10 min, and the first 5 min was regarded as environmental stabilization in the scanner. This was needed to avoid drift in the baseline in some animals,

as shown in Figure 5(f). Longer baseline scan time is recommended in future studies to remove this drift. Despite animal constraints, motions during the experiment may also affect the DGE signal, especially during the glucose infusion. In this study, image registration was adopted to minimize the undesirable effects induced by motions, and this issue can be further relieved by using a more motion insensitive acquisition module, such as ultrashort echo time (UTE)-CEST.⁵⁸ The clinical translation of onVDMP is of great importance but may face some challenges, such as B_0 drift, specific absorption rate (SAR) issue, and limited D-glucose dose for human study. The B_0 issue is being addressed, for instance in Herz et al., by adopting an additional B_0 correction module specified.⁵⁹ The SAR for the onVDMP sequence can be tuned by increasing the mixing time during the binomial pulse and we currently already have a first version on the human scanner. Actually, it has been shown that with the use of dual transmit body coils, SAR can be kept sufficiently low even during CW-CEST.⁶⁰ The limited D-glucose dose for human study (25 g normal D-glucose in a 50-mL sterile water solution)⁶¹ may challenge the sensitivity and specificity of onVDMP MRI, and further validation is required.

Conclusions

An onVDMP MRI sequence with a long saturation module was applied to study D-glucose uptake in mouse brain parenchyma and CSF. The feasibility of discriminating AD and WT mice was demonstrated. The results indicate that the AD mice with tau pathology have smaller D-glucose uptake in the parenchyma and slower D-glucose uptake in CSF compared to their WT counterpart, which was tentatively attributed to reduced glucose transporter density or impaired transporter functioning at BBB and BCSFB of AD mice. The results also suggest that the DGE curve of CSF has the potential to be a non-invasive tool for assessing the glymphatic function in brain.

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The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

LC and JX designed and performed experiments, processed data, and wrote the manuscript; PCMVZ helped with designing the study, interpreting the data, and manuscript editing; ZW performed experiments, measured mouse brain volumes, and body weight; KWYC, YL, KS, SB, JH, XX, and HL interpreted the data; and PCW and TL provided AD mouse model and interpreted the data. All authors discussed the results and commented on the manuscript.

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Supplemental material

Supplemental material for this article is available online.

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ARTICLE



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OPEN

In vivo imaging of phosphocreatine with artificial neural networks

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Phosphocreatine (PCr) plays a vital role in neuron and myocyte energy homeostasis. Currently, there are no routine diagnostic tests to noninvasively map PCr distribution with clinically relevant spatial resolution and scan time. Here, we demonstrate that artificial neural network-based chemical exchange saturation transfer (ANNCEST) can be used to rapidly quantify PCr concentration with robust immunity to commonly seen MRI interferences. High-quality PCr mapping of human skeletal muscle, as well as the information of exchange rate, magnetic field and radio-frequency transmission inhomogeneities, can be obtained within 1.5 min on a 3 T standard MRI scanner using ANNCEST. For further validation, we apply ANNCEST to measure the PCr concentrations in exercised skeletal muscle. The ANNCEST outcomes strongly correlate with those from ³¹P magnetic resonance spectroscopy ($R = 0.813$, $p < 0.001$, t test). These results suggest that ANNCEST has potential as a cost-effective and widely available method for measuring PCr and diagnosing related diseases.

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Phosphocreatine (PCr) is a high-energy phosphate compound that is abundant in muscle and brain and used by creatine kinase isoenzymes to generate adenosine triphosphate from adenosine diphosphate. PCr plays a vital role in cellular energy buffering and energy transport, particularly in tissues with high and fluctuating energy demands, such as skeletal muscle, cardiac muscle, and brain¹. The measurement of PCr provides a unique way to achieve insight into cellular energetics, and has shown great potential in many areas, such as for evaluating mitochondrial function *in vivo*², and for identifying peripheral arterial disease³ and heart failure⁴. PCr concentrations are reduced in several neurodegenerative and muscle diseases. To date, phosphorus-31 magnetic resonance spectroscopy (³¹P MRS) has been the established method for noninvasively detecting and quantifying PCr *in vivo*^{2,5,6}. In addition to PCr measurement, ³¹P MRS also provides information about pH, inorganic phosphate, and adenosine phosphates (ATP, ADP, and AMP) in tissue. In practice, ³¹P MRS is most commonly applied to monitor the time dependencies of pH and PCr variation during exercise and recovery for assessing mitochondria^{7,8}. However, the inherent low detection sensitivity of ³¹P MRS results in low spatial resolution and long acquisition times that hinder its wide application. In addition, ³¹P MRS is not available on most magnetic resonance imaging (MRI) scanners in clinical practice due to additional hardware costs associated with ³¹P excitation/detection that are not used in clinical ¹H MRI scanners, the significant expense of broadband transmitting/receiving hardware, and the impracticality of having to switch coils in a clinical setting. Therefore, currently, there are no routine diagnostic tests to noninvasively quantify or map the distribution of PCr in tissue with clinically relevant spatial resolution and scan time.

Chemical exchange saturation transfer (CEST) is an MRI sensitivity-enhancing approach that exploits the interaction between exchangeable protons in low-concentration molecules and the water protons detected in MRI, which has shown great potential in detecting various metabolites *in vivo*^{9–11}. CEST MRI does not require special system hardware or coils, and thus can be performed on standard clinical MRI scanners. However, its translation from high-field animal studies to clinical practice has been slowed by the lower field strengths used in the clinic (1.5 T and 3 T), at which the frequency shifts between the exchangeable protons and the water resonance, as well as the contrast-to-noise ratio of the CEST signal, are reduced. Currently, no prior studies have yet been performed to experimentally evaluate human PCr mapping on clinical MRI systems. To explore and develop high-quality PCr mapping using CEST for clinical practice at low field strengths, optimization of the PCr CEST acquisition is required, and the quantification method needs to be robust against the inevitable static magnetic field (B_0) and radio-frequency transmit field (B_1) inhomogeneities, as well as interference from other saturation transfer components in tissues.

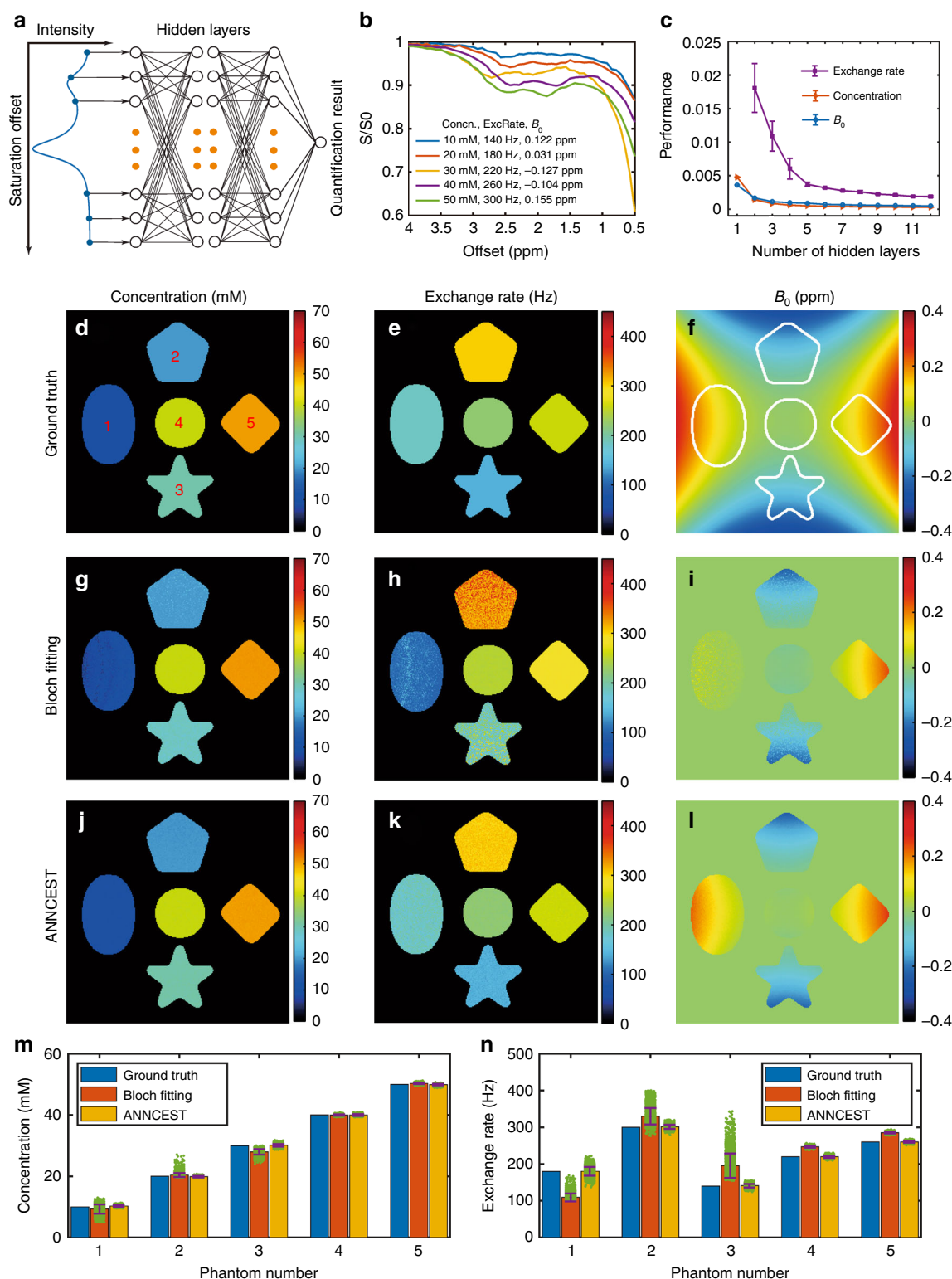
Artificial neural networks (ANNs) are increasingly used in many diverse areas^{12–14} to successfully extract relevant features from extremely large, annotated data sets, and utilize them to create predictive tools based on patterns hidden inside. Once trained, ANNs can apply the learned knowledge to analyses of other data and/or solve task-specific problems. In this study, we demonstrate that ANNs can be used for CEST quantification, dubbed hereafter as ANNCEST. More specifically, we show that this trained neural network can accurately and simultaneously predict multiple important properties, including metabolite concentration, the exchange rate of the exchangeable protons, and B_1/B_0 homogeneity information, with just the simple input of a Z spectrum (water saturation transfer spectrum) for each image volume element (voxel). After first training and validating ANNCEST using numerical simulations and PCr phantom data

at 3 T, we optimize the PCr CEST acquisition to obtain maximum PCr contrast on human skeletal muscle, and again train and apply ANNCEST. We then show the feasibility of applying ANNCEST to simultaneously quantify the PCr concentration of human skeletal muscle, the exchange rate of the guanidinium protons from PCr, and the B_0 and B_1 maps on a clinical 3 T MRI scanner. As additional validation, the PCr depletion and recovery in exercised human skeletal muscle were detected and quantified by ANNCEST, and the results were compared with those from ³¹P 2D MRS. We also discuss the potential applications of ANNCEST as well as its advantages and limitations. The results suggest that the exchangeable guanidinium protons of millimolar concentration PCr can be exploited to detect it via the water signal in MRI with greatly enhanced sensitivity (molar signal) using CEST MRI, and its concentration can be quantified using ANNs.

Results

Validation of ANNCEST. We implemented ANNCEST with a feed-forward neural network as shown in Fig. 1a. The neural network was trained using Z spectra generated by the Bloch–McConnell equations¹⁵ for various concentrations and exchange rates of exchangeable protons at multiple offset frequencies, and for spatially varying B_1 and B_0 . Gaussian white noise was added to the training Z spectra to mimic the real situation (Fig. 1b). Numerical simulations were performed to validate that ANNCEST can accurately and simultaneously predict metabolite concentration, exchange rate of exchangeable protons, and B_0 with the simple input of a Z spectrum per voxel. The simulated Z spectra were generated based on a PCr phantom model at 3T containing two CEST peaks at 1.95 ppm and 2.5 ppm as shown in Supplementary Fig. 1d–f. The exchange rate ratio between 1.95 ppm and 2.5 ppm was set to 1:2.19 according to measures obtained in a PCr phantom at 37 °C using an inversion recovery technique on a 17.6 T NMR spectrometer (see Supplementary Section 2 and Supplementary Fig. 2). The number of hidden layers was optimized to avoid the risk of overfitting (Fig. 1c), and the initial findings suggested that seven hidden layers can provide adequate predicted capacity, because the improvement of performance with more hidden layers was less than 5×10^{-4} . Therefore, seven hidden layers were used in this study unless otherwise specified. The statistical analyses of the training results are given in the Supplementary Section 3. To test the performance of the trained neural network for quantifying new data, we generate new Z spectra pixel by pixel based on the ground-truth maps shown in Fig. 1d–f. To give a comparison to ANNCEST, Bloch equation fitting was also performed. The quantification results and statistical analysis in Fig. 1g–n show that ANNCEST can yield better fidelity to ground truth compared with Bloch equation fitting, especially for the quantification of exchange rate and B_0 in regions with low concentration (detailed values are listed in Supplementary Table 1). It should be noted that ANNCEST is completed within 2 s on a personal computer (Intel i5-6300U CPU with 8 G memory), while the Bloch fitting requires around 18 h on a cluster computer with 8 parallel computing (AMD Opteron 6100 8-core CUP with 16 G memory).

The same neural networks were applied to quantify the Z-spectra obtained from PCr phantoms, and the results are shown in Fig. 2. An excellent correlation ($R = 0.9989$) was observed between the ground truth and predicted phantom PCr concentration. The related Bland–Altman analysis of concentration is shown in Fig. 2f. The exchange rates obtained by ANNCEST were consistent with those obtained using an inversion recovery approach (260 ± 40 Hz, mean $\pm$ s.d.) (detailed values are listed in Supplementary Table 2). The predicted B_0 map (Fig. 2e) showed a strong correlation ($R = 0.9969$) with that obtained by water



saturation shift referencing (WASSR) MRI¹⁶, as illustrated in Fig. 2h.

Optimize PCr CEST contrast in vivo on human skeletal muscle at 3 T. Our previous study on mouse skeletal muscle at 11.7 T showed that PCr has two distinct CEST peaks around 1.95 ppm and 2.5 ppm, and that the 1.95-ppm peak also contains contributions from Cr and protein guanidinium protons¹⁷. However, the CEST line shapes can be significantly different at lower fields,

especially in vivo, where strong magnetization transfer contrast (MTC) exists. Here, CEST experiments with different saturation powers and saturation lengths were performed on human skeletal muscle to experimentally evaluate the PCr contrast on a 3 T clinical system. Due to the presence of MTC, the Z spectra of human skeletal muscle are quite different from those obtained on PCr phantoms (see Supplementary Fig. 1). From the optimization results for saturation powers ranging from 0.2 μ T to 0.8 μ T (Fig. 3a–d), the Z spectrum obtained with a relatively low

Fig. 1 Validation of ANNCEST with numerical simulation. **a** Diagrammatic representation of the feed-forward artificial neural network used in this study. This network comprises three layers: an input layer, fully connected hidden layers, and an output layer. The input of the neural network is the intensity of the Z spectrum at different frequency offsets, and the outputs are the predicted values of metabolite concentrations, exchange rates of exchangeable protons, and B_1/B_0 . **b** Representative Z spectra of PCr phantom generated by three-pool Bloch–McConnell equations. Z spectra from 0.5 to 4.0 ppm were sampled with a 50-saturation offset over equal intervals. Gaussian white noise with a standard deviation of 0.35% and B_0 inhomogeneity offsets were added to the Z spectra. The saturation power and length were set to 0.6 μ T and 10 s, respectively. **c** The performance of neural networks as a function of the number of hidden layers. The error bar was obtained by repeating the neural network training five times. **d–f** The ground truth maps of concentration, exchange rate at 2.5 ppm, and B_0 for generating validated Z spectra. The matrix size of maps is 256×256 . The maps of concentration, exchange rate, and B_0 obtained by Bloch equation fitting (**g, h**) and ANNCEST (**j–l**), respectively. **m, n** Quantified concentrations and exchange rates, respectively. The bar and error bar indicate the mean value and standard deviation across each phantom, respectively ($n = 4523, 3655, 2770, 2610$, and 2790 pixels for phantom numbers 1–5, respectively).

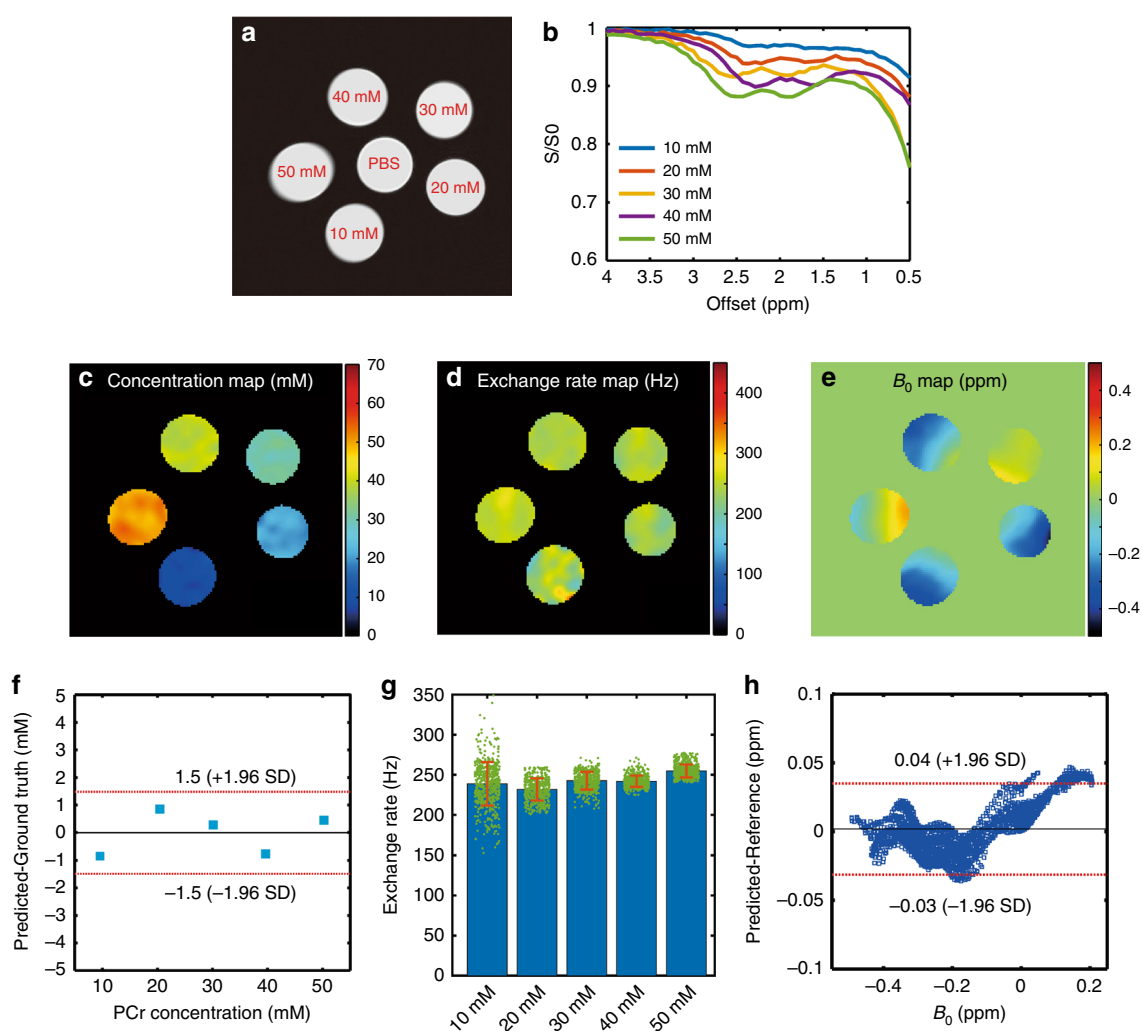


Fig. 2 Validation of ANNCEST at 3 T (preclinical MRI) on a phantom consisting of test tubes with different concentrations of PCr. All experiments were performed at 37 °C. **a** The arrangement of the PCr phantoms with different concentrations. **b** Representative Z-spectra extracted from one pixel of each of the PCr phantoms. The ANNCEST-predicted concentration (**c**), exchange rate at 2.5 ppm (**d**), and B_0 maps (**e**) for the CEST experiments collected using a 10-s saturation pulse of 0.6 μ T. The neural network is identical to the one used in numerical simulation. **f** Bland–Altman plot for the predicted concentration and ground truth. **g** The exchange rate quantification results. The bar and error bar indicate the mean value and standard deviation across each phantom, respectively ($n = 579, 473, 508, 587$, and 566 voxels for tubes with concentration from 10 mM to 50 mM, respectively). The ground truth of exchange rate (260 ± 40 Hz) was obtained using inversion recovery technique as specified in Supplementary Materials. **h** Bland–Altman plot for the predicted B_0 map and referenced B_0 map obtained via WASSR method.

saturation power of 0.2 μ T exhibits two discernible CEST peaks around 2.5 ppm and 1.95 ppm. With the increase in saturation power, the observed CEST signal (ΔZ) at 2.5 ppm increases at first and then decays after reaching a maximum at 0.6 μ T. The CEST peak at 2 ppm is indiscernible from the Z spectra with the saturation power larger than 0.4 μ T due to the MTC scale-down

effect¹⁸. The saturation length was optimized with a fixed saturation power of 0.6 μ T. From the quantitative analysis shown in Fig. 3i, a CEST protocol with a saturation length of 800 ms yields the maximum ΔZ . Therefore, a saturation power of 0.6 μ T and a saturation length of 800 ms were applied in the remaining studies unless otherwise specified.

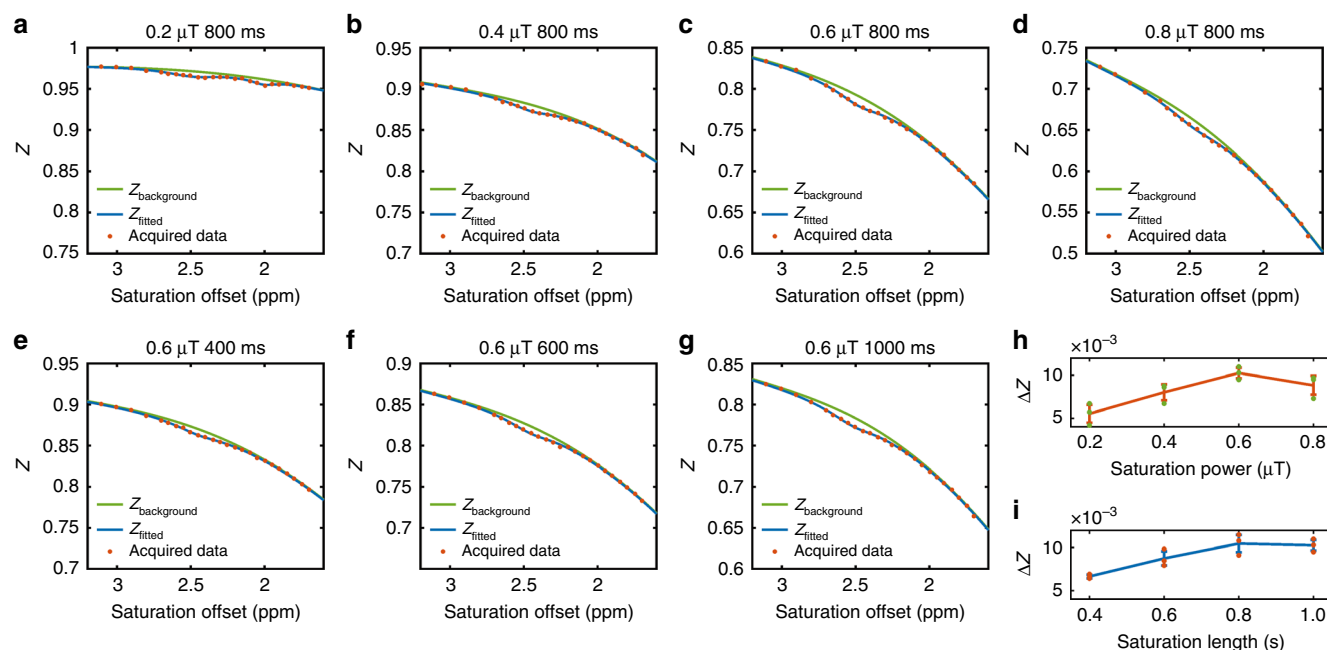


Fig. 3 Optimization of PCr CEST contrast in human skeletal muscle on a 3 T clinical scanner. The Z spectra were fitted using a polynomial and Lorentzian line-shape fitting (PLOF) CEST method. The observed CEST signal (ΔZ) indicating the difference between CEST peak and fitted background at 2.5 ppm is used as the evaluation parameter. **a–d** Acquired data and fitted results of CEST experiments with fixed saturation length of 800 ms and four saturation powers. **e, f** Acquired data and fitted results of CEST experiments with fixed saturation power of 0.6 μT and different saturation lengths. **h, i** Quantitative analysis of ΔZ with different saturation powers and saturation lengths, respectively ($n = 3$ ROIs). Error bars show standard deviations in ROIs.

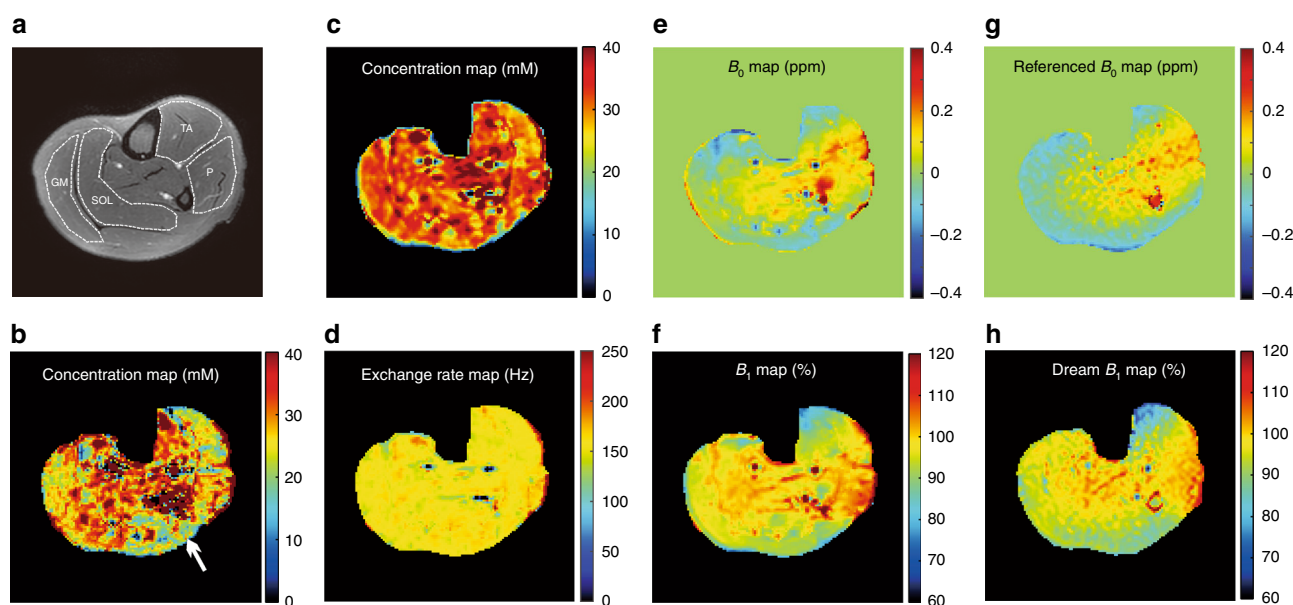


Fig. 4 PCr mapping on human skeletal muscle using ANNCEST. **a** A high-resolution T_2 weighted anatomy image with segmentation of gastrocnemius medial (GM), soleus (SOL), tibialis anterior (TA), and peroneus (P). **b** Concentration map obtained using the PLOF method. The PCr concentration (**c**) and exchange rate (**d**) maps together with the B_0 (**e**) and B_1 (**f**) maps obtained by the ANNCEST method using the CEST images acquired with 0.6 μT saturation power and 800 ms saturation length. The B_1 map was normalized by the reference power 0.6 $\mu\text{T} = 100\%$. Reference B_0 (**g**) and B_1 (**h**) maps of the same slice obtained by the established dual-echo²² and DREAM²³ methods, respectively.

High-quality PCr mapping on human skeletal muscle using ANNCEST. To train the in vivo network, Z spectra of human skeletal muscle at 3 T were generated assuming the experimentally verified situation of a single PCr CEST peak at 2.5 ppm and an additional broad background signal to account for the contributions from MTC and other metabolites that do not show distinct CEST peaks on the Z spectrum. Maps of concentration,

exchange rate, B_0 and B_1 obtained by applying the newly trained neural network to the in vivo data are shown in Fig. 4c–f. The previously published polynomial and Lorentzian line-shape fitting (PLOF) method was performed for comparison^{17,18}. The results showed that the concentration map obtained by PLOF was degraded due to inherent B_0 and B_1 inhomogeneities as indicated by the white arrow (Fig. 4b), while the concentration map

obtained by ANNCEST shows much better robustness against B_0 and B_1 inhomogeneities. The quantified PCr concentrations of gastrocnemius medial (GM), soleus (SOL), tibialis anterior (TA) and peroneus (P) over five volunteers were 31.9 ± 2.0 mM, 31.7 ± 3.3 mM, 30.8 ± 4.1 mM, and 30.9 ± 3.9 mM, consistent with previous total muscle reports (29–36 mM)^{19,20}. The quantified exchange rate (164 ± 36.8 Hz across muscle) is consistent with a previously reported value (140 ± 50 Hz)²¹. The B_0 and B_1 maps obtained by ANNCEST also show high similarity with those obtained by the established individual methods of dual-echo²² for B_0 and DREAM²³ for B_1 . Although B_0 and B_1 showed clear variation in the muscle (B_0 : -0.15 – 0.25 ppm; B_1 : 0.6 – 0.8 μ T), the ANNCEST method was still able to provide homogeneous concentration and exchange rate maps across the muscle, except for the regions with blood vessels. In order to validate the robustness of ANNCEST against T_1 and T_2 variations, we applied trained ANNCEST to quantify Z-spectra simulated with different water T_1 and T_2 values²⁴ (Supplementary Fig. 10). From Supplementary Fig. 10b, when increasing water T_1 over a large range from 1.0 s to 2.0 s, the quantified concentration increased only from 35.03 ± 1.95 mM to 37.33 ± 1.90 mM, in an approximately linear fashion. The exchange rate obtained by ANNCEST possessed excellent resistance against water T_1 variation (160.7 ± 2.6 Hz at $T_1 = 1$ s vs. 159.0 ± 3.3 Hz at $T_1 = 2$ s), as shown in Supplementary Fig. 10c. The quantified concentrations and exchange rates as a function of water T_2 are shown in Supplementary Fig. 10e, f. These results indicate that ANNCEST still can yield reasonable accuracy when water T_2 varies from 15 to 50 ms. We then performed a similar test for sensitivity of ANNCEST to T_1 and T_2 variation for the PCr protons. The results in Supplementary Section 8 and Supplementary Fig. 11 show that concentrations and exchange rates possess excellent resistance against T_1 variation of PCr proton (34.75 ± 2.35 mM and 163.7 ± 3.5 Hz at 30 ms vs 36.06 ± 2.16 mM and 158.6 ± 4.9 Hz at 70 ms). Similarly, ANNCEST still can yield satisfactory accuracy when the PCr proton T_2 varies from 15 ms to 25 ms, giving a concentration variation between 34.9 mM and 36.9 mM, while the exchange rate ranges from 162.8 Hz to 159.0 Hz.

The PCr mapping using ANNCEST was further validated by comparison with ^{31}P 2D MRS measures obtained before and during in-magnet plantar flexion exercise. Subjects underwent the same exercise protocol, once with CEST acquisitions and once with ^{31}P 2D MRS, in varied order. Shortly after the exercise, the PCr depletion in the gastrocnemius muscles recovered to basal values on the PCr maps obtained by both ANNCEST and ^{31}P 2D MRS as shown by the representative results in Fig. 5b, c. The PCr recovery time can in principle be obtained from the dynamic PCr maps as demonstrated in Supplementary Section 5. The Z-spectra for a region of interest in the gastrocnemius muscles obtained at two-time points are also plotted and demonstrate the decrease of the PCr CEST peak during the exercise (Fig. 5d, e), namely from 30.38 mM to 15.42 mM. The PCr depletion observed in the different muscle regions is in good agreement with those reported previously in healthy volunteers using ^{31}P spectroscopy, in which the gastrocnemius muscle showed significantly greater PCr depletion than other muscle groups during plantar flexion exercise^{25–27}. PCr concentrations obtained by ANNCEST during exercise agreed very well with those measured by ^{31}P 2D MRS, as indicated by the correlation ($p < 0.001$, Student's t test) and Bland–Altman analyses between two methods in Fig. 5f, g, respectively.

Discussion

PCr is an important intracellular high-energy phosphate molecule that is depleted in many neurologic and muscle diseases but one

that is rarely quantified or mapped noninvasively in routine clinical practice. Here, we present a technique, named ANNCEST, to reliably detect, quantify, and image high-quality PCr distribution of human skeletal muscle on a 3 T standard clinical MRI scanner within 1.5 min.

Our previous studies at high magnetic field strength (11.7 T)¹⁷ showed that PCr has two CEST peaks at 1.95 ppm and 2.5 ppm. The same observation was confirmed by another group using 9.4 T and 15.2 T MRI²⁸. However, to the best of our knowledge, there has been no published study to map PCr in human skeletal muscle with CEST at clinical magnetic field strength (1.5 T or 3 T). The observed PCr CEST signal at low magnetic field strength is significantly different from that at high fields due to a reduced frequency difference between the exchangeable protons and water protons. From the optimization results shown in Fig. 3, two discernible CEST peaks at 1.95 ppm and 2.5 ppm can be observed for low saturation power (0.2 μ T). However, with increased saturation power, the CEST peak at 1.95 ppm becomes indiscernible and only one CEST peak at 2.5 ppm remains. This is fortunate for PCr quantification because the CEST peak at 2.5 ppm is dominated by PCr as validated previously^{17,28}. From the PCr phantom experiments shown in Fig. 2, due to the reduced CEST contrast, the exchange rate of 10 mM PCr phantom is more vulnerable to systematic imperfections and exhibits a larger standard deviation compared with the others. Therefore, optimizing CEST sequence to obtain maximum PCr contrast is critical for robust quantification since the PCr contrast in vivo is around 1% (Fig. 3h, i). The quantitation of the metabolites and proteins that form the Z-spectrum is challenging in CEST studies due to the concurrence of the CEST signals from solid-like macromolecules and mobile proteins, as well as the water direct saturation. Many techniques have been proposed for quantification in CEST studies. The most common one is the asymmetry analysis method, i.e., subtracting images acquired at two symmetric offsets with respect to the water resonance, which has been used for quantifying various metabolites^{29–31}. Some other methods such as the Lorentzian fitting method³² and the rotating frame relaxation theory^{33,34} have also been proposed for quantification. In most of the CEST quantification methods, the contribution of the CEST signal in the Z-spectrum was usually simplified to a linear function and all contributions were assumed to be superimposed linearly; however, these simplified models usually only work in certain situations, such as with weak saturation powers. CEST quantification is further complicated by the inevitable B_0 and/or B_1 inhomogeneities, which can shift or distort the in vivo CEST Z-spectra. Usually, B_1 and B_0 maps need to be collected and included in the CEST quantitation modeling, which not only lengthens the CEST experimental time but also makes the quantification of CEST more challenging. Here, we present the first evidence that ANNs can be used for quantifying in vivo CEST signal. The initial idea of this study was inspired by the work of Bo Zhu et al.¹⁴, who demonstrated that conventional image reconstruction methods can be replaced by ANN-based methods with much-improved performance. Similar to image reconstruction, the CEST quantification is a kind of inverse problem, where the useful information (e.g., concentration and exchange rate) needs to be decoded from the acquired data (i.e., the intensities in a Z-spectrum). The encoding process of CEST MRI can be well described by the Bloch–McConnell equations and a training Z-spectrum can be easily generated with known parameters. However, due to the complexity of the Bloch–McConnell equations, an accurate solution is hard to derive especially with the presence of possible B_0 and B_1 effects, which means decoding quantitative concentrations and exchange rates from Z-spectra is challenging. Here, ANNCEST provides a new dimension for CEST quantification. A fully connected feed-

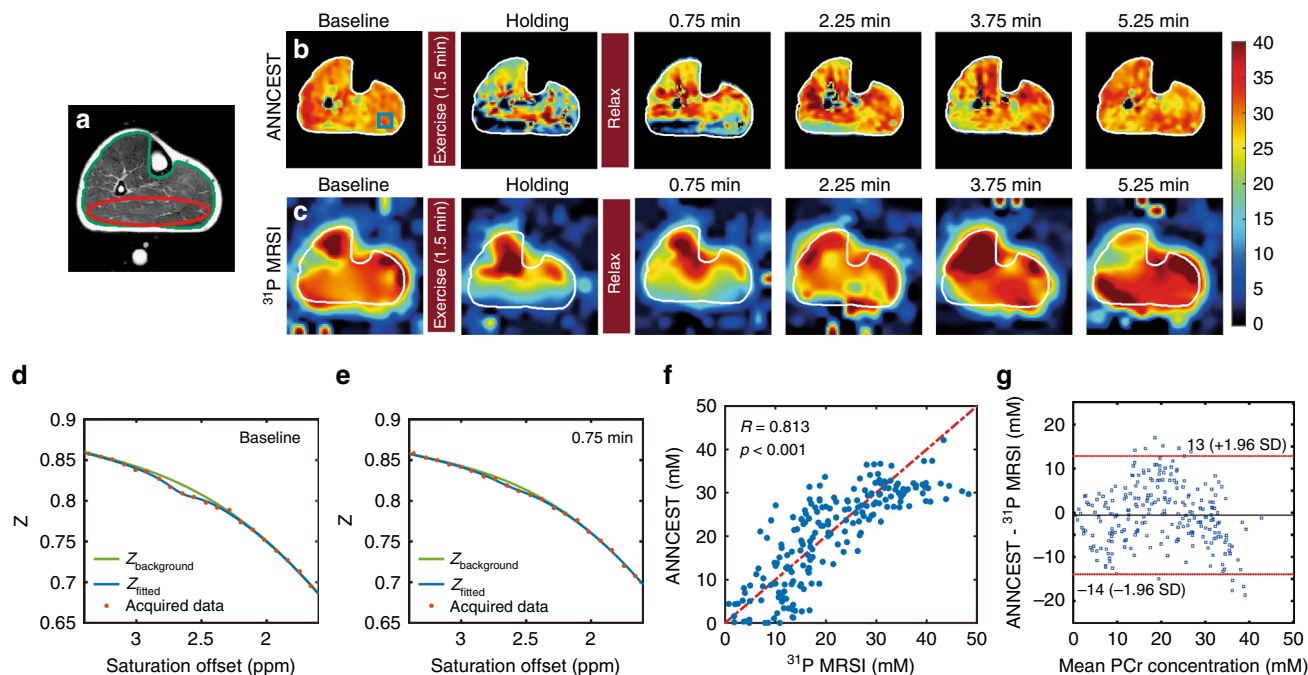


Fig. 5 Representative PCr maps of human skeletal muscle pre and post in-magnet plantar flexion exercise using ^{31}P 2D MRS and ANNCEST methods. **a** T_2 -weighted anatomy image. PCr concentration maps obtained by **b** ANNCEST and **c** ^{31}P 2D MRS during the whole exercise process. The Z spectrum at baseline (**d**) and post exercise (**e**) extracted from the blue rectangle region shown in **b**. **f** The correlation analysis of ANNCEST and ^{31}P 2D MRS from the red ellipse region shown in **a** for all four subjects ($n = 202$ voxels). **g** Bland-Altman plot for the PCr concentrations obtained by ANNCEST and ^{31}P 2D MRS.

forward neural network with enough neurons in its hidden layer is known to be good at finding patterns behind one-dimensional data³⁵, which is well suited for CEST quantification. Instead of needing to derive the solution of the Bloch–McConnell equations, we train the ANN with a large annotated dataset to extract the relationship between the Z-spectrum and quantifiable parameters. As shown in Supplementary Section 4, the depth, width, and offset of in vivo PCr peak are related to concentration, exchange rate, and B_0 introduced frequency shift, respectively, while B_1 variation affects overall Z-spectral background intensity (Supplementary Fig. 5d). These effects can be fully exploited by ANN and applied to simultaneously quantify these parameters (Supplementary Figs. 3 and 4). The ANNCEST approach proposed here provides an additional dimension for CEST quantification. Similar to other artificial intelligence methods, ANNCEST is a data-driven quantification method and its accuracy highly depends on the training data. In this study, the training data were generated using the Bloch–McConnell equations with consideration of B_0 and B_1 inhomogeneities and noise. The relatively homogeneous T_1 and MTC across the human skeletal muscle benefit the generation of training data^{24,36,37}. With optimal saturation parameters, training Z-spectra within a limited spectral range (1.3 ppm–3.5 ppm) can be generated using a three-pool Bloch McConnell simulation, namely water protons, PCr guanidinium protons, and background. The background including the contributions from MTC and all other metabolites can be well represented by a single pool (Supplementary Fig. 5e). The discernible PCr guanidinium CEST peak in vivo provides a unique opportunity for ANNCEST to learn the relationships between the Z spectrum and PCr concentration, exchange rate, and B_0 and B_1 (Supplementary Sections 3 and 4), and to apply the learned knowledge to simultaneously quantify these multiple parameters, as demonstrated in Figs. 4, 5. Previous studies^{2,37,38} have shown that the pH, T_1 , and T_2 can change after exercise. We included the effects of pH and T_2 on training data by adopting varying

exchange rates and T_2 values. In addition, we tested the performance of ANNCEST using simulated data over a range of water and PCr proton T_1 and T_2 values (Supplementary Figs. 10 and 11). The results showed that the ANNCEST-determined exchange rates and concentrations are robust over a water T_1 range from 1.0 s to 2.0 s and a water T_2 range from 15 ms to 50 ms, which are the relevant ones of in vivo ranges²⁴. Concentrations were also very insensitive to the PCr proton T_1 and T_2 . ANNCEST can be applied to quantify other metabolites under different situations (see Supplementary Figs. 7, 8 and 9). In the case where the Z-spectrum may be difficult to simulate using Bloch–McConnell equations, the training data can be generated by combining acquired Z-spectra with corresponding quantification results obtained through other gold standard methods. An advantage of CEST MRI for collecting adequate training data is that the size of the training data set is proportional to the number of pixels of the CEST image since each pixel has its own Z spectrum, thus providing sufficient samples and enhancing the power of the resulting data (see Supplementary Section 7).

Though ANNCEST can efficiently exploit the relationship between the Z spectrum and different parameters, the range and frequency offset interval of the Z spectrum and the quantifiable parameters still need to be carefully designed for the successful application of ANNCEST. For the numerical simulations and phantom experiments, the frequency offsets of the Z-spectra ranged from 0.5 to 4 ppm with a total offset number of 50. Within this limited Z-spectral range, B_1 and concentration both affect the depth of CEST peak. Therefore, when varying B_1 variation (0.5 μT –0.7 μT) in the training, the accuracy of concentration quantification was significantly degraded (the linear regression R -value of neural network training dropped from 0.99983 to 0.85054). In this study, due to the relatively small FOV of phantom experiments ($30 \times 30 \text{ mm}^2$), we, therefore, assumed the B_1 to be homogeneous across phantoms and a fixed B_1 was adopted in the training Z-spectra for numerical simulation and phantom

experiments. This assumption worked well, and high fidelity of PCr concentrations was obtained, as shown in Fig. 2c, f. In phantom experiments where B_1 inhomogeneity cannot be neglected, an additional range of Z-spectrum (e.g., the direct saturation of water or more background regions) may be required for accurate quantification of PCr concentration, as well as B_1 . For the human experiments, successful quantification of B_1 and concentration was possible since B_1 affects not only the PCr CEST peak but also the MTC-dominant background. An ANN can well exploit this additional information and provide accurate quantification of B_1 and concentration as shown in Supplementary Fig. 4g, h. Compared with MRS methods for measuring PCr metabolism of skeletal muscle on clinical scanners such as ^{13}C MRS³⁹ and ^{31}P MRS^{2,5,40–42}, ANNCEST doesn't require any special MRI transmit or detection coils for heteronuclear or expensive additional system hardware (amplifiers and cabling) and is ready for use on most clinical MRI scanners. In addition, CEST does not require the administration of exogenous contrast agents. With a well-trained neural network, the PCr maps can be calculated rapidly (within a few seconds) from the acquired CEST images, which is much more time-efficient than existing fitting methods, such as Bloch fitting (within a few hours) and PLOF method (within a few minutes), and can provide real-time analysis of a CEST experiment. This is important for the future clinical applications of PCr mapping, where ANNCEST has the potential to address the major target of mapping of the time dependencies of PCr concentration during exercise and recovery. A preliminary result of estimating spatially resolved map of PCr recovery rate constant using ANNCEST is shown in Supplementary Fig. 6 and a recovery time constant of 70.7 ± 55.4 s was obtained, which is consistent with that reported in the previous study (63.1 ± 25.9 s)⁴³. However, the temporal resolution of PCr ANNCEST in the current study (i.e., 90 s) was too low to capture very detailed dynamic changes. Future possibilities for reducing the scan time of PCr ANNCEST are adopting fewer saturation offsets or utilizing fast CEST sequences^{44–46}, which needs further study. Finally, as can be seen from Supplementary Fig. 2d, e, the exchange rate depends on both pH and temperature. While we fit out the exchange rate, it is currently not trivial to separate out the effects of pH and temperature and this will be a topic for further study.

In conclusion, as a noninvasive and high-spatial-resolution PCr mapping technique that can be implemented on widely available clinical MRI scanners, PCr ANNCEST has tremendous potential to bring non-contrast metabolic imaging to the clinic whereby identifying, quantifying, and mapping metabolic changes at rest and exercise may guide the diagnosis of many muscle-related, neurologic, and other diseases.

Methods

Neural network architecture and training. A feed-forward neural network composed of one input layer, one output layer, and multiple hidden layers was used for the current study (Fig. 1). The input of the neural network is Z-spectral intensity at different saturation offsets, and the output is the corresponding quantification result. A sigmoid transfer function is used in the hidden layer, and the scales of input and output are normalized to $[-1, 1]$ using a linear function. The weights and biases of the neural network are trained by a scaled conjugate gradient backpropagation algorithm. Some strategies were utilized to avoid overfitting. First, the number of hidden layers was optimized to provide just large enough fitting capacity for the current study. Second, early stopping was applied during the training process. The training data were randomly divided into three sets: training (80%), validation (15%), and test (5%) data. The training data were used to calculate the gradient and update the weights and biases of the neural network. The error on validation data was monitored during the training process. If the validation error increased for 40 iterations, the training was stopped. The test data were used to evaluate the randomness of data division. The test set was designed to compare the performance of different ANN models, and while the error on the test set is not used during training, in practice, it is still useful to monitor. If the error in the test set reaches a minimum at a significantly different

iteration number than the error in the validation set, this might indicate a poor division of the data set. In this study, since the training Z-spectra were generated by randomizing the quantifiable parameters within certain ranges and no other ANN model was adopted, the test set is not critical for the training of neural network. Therefore, we set the portions of the test set and the training set to 5% and 80%, respectively. Finally, a modified performance function, $\gamma \times msw + (1 - \gamma) \times mse$, was used to evaluate the neural network, where mse refers to the mean squared normalized error in training data, msw stands for the mean of the sum of squares of the network weights and biases, and γ is the regularization parameter (set to 0.01 in the current study). The application of msw causes the network to have smaller weights and biases, and forces the network response to be smoother and less likely to overfit. Besides the early stopping constraint, the neural network training was stopped once any of the following conditions were met: (1) the calculated gradient was smaller than 10^{-7} ; (2) the mean squared normalized error on validation data was smaller than 10^{-4} ; (3) or the maximum epochs number reached 10^6 . All of the above processing was accomplished on the MATLAB platform (www.mathworks.com, version 9.4.0.813654).

Z spectra for neural network training. In this study, training data were generated using the Bloch–McConnell equations with consideration of various imperfect situations, such as B_0/B_1 inhomogeneity and noise. Details of the parameters used can be found in Supplementary Table 3 for the phantom and Supplementary Table 4 for the human leg. To mimic the real situation, measurements of water T_1 and T_2 values were performed for use in this training data. However, it is important to realize that such measurements are not necessary for the ANNCEST applications after the neural network is well trained (See Results Section above). For the numerical simulation and phantom experiments, the frequency offsets of the Z-spectra ranged from 0.5 to 4 ppm with a total offset number of 50. The offsets of PCr CEST peaks were set to 1.95 ppm and 2.5 ppm. The exchange rate ratio between 1.95 ppm and 2.5 ppm was set to 1: 2.19 according to the measurement in a PCr phantom at 37° using a magnetization recovery technique (see Supplementary Section 2). The T_1 and T_2 of water protons were set to 2.6 s and 1.8 s, respectively, according to the measurements on the phantom. The T_1 and T_2 values for PCr protons were set to 0.05 s and 0.02 s, respectively. The saturation power and duration were 0.6 μT and 10 s. The concentration, exchange rate at 1.95 ppm, and B_0 inhomogeneity were randomly chosen from the ranges of 5 to 85 mM, 50 to 200 Hz, -0.4 to 0.4 ppm, respectively. Gaussian white noise with zero mean value and 0.0015 standard deviations was imposed on the simulated Z spectra. The number of Z-spectra used for neural network training was 10^5 .

For the PCr mapping of human muscle, the frequency offset range of the acquired Z spectra was from 1.3 to 3.5 ppm, with a total offset number of 50. The CEST peak offset was set to 2.5 ppm. The T_1 was set to 1.2 s according to the measurement obtained on human skeletal muscle. T_2 was varied from 15 to 35 ms. The T_1 and T_2 values for PCr protons were set to 0.05 s and 0.02 s, respectively. The robustness of ANNCEST against T_1 and T_2 variations of PCr protons is shown in Supplementary Fig. 11. The saturation power and duration were, respectively, 0.6 μT and 800 ms. The concentration, exchange rate, B_0 inhomogeneity, and B_1 inhomogeneity were randomly chosen from the ranges of 0 to 100 mM, 80 to 230 Hz, -0.25 to 0.25 ppm, and 0.5 to 0.7 μT , respectively. Gaussian white noise with a zero mean value and 0.0035 standard deviations was imposed on the simulated Z-spectra. The MTC-dominated background signal was incorporated as an additional pool with a concentration of 8 M for the exchanging protons and an exchange rate of 30 Hz. The T_1 and T_2 values for the background signal were set to 1 s and 9.1×10^{-6} s, respectively. The lineshape for the background signal was a Super Lorentzian function. The goodness of background fitting using the above parameters is shown in Supplementary Fig. 5e. The number of Z-spectra used for neural network training was 10^5 .

Numerical simulation. The ground truth concentration, exchange rate, and B_0 maps with a matrix size of 256×256 are shown in Fig. 1d–f. The Bloch equations were applied to simulate the Z-spectrum for each pixel. In comparison to ANNCEST, Bloch fitting was performed to quantify the concentration, exchange rates, and B_0 . The initial values for the ranges of concentration, exchange rate at 1.95 ppm, and B_0 were 5 mM (5–85 mM), 50 Hz (50–200 Hz), and 0 ppm (-0.4 to 0.4 ppm), respectively. The other parameters were the same as those used for the Z-spectra simulation. The Bloch fitting was performed at a Penguin computer cluster with eight parallel workers (AMD Opteron 6100 8-core CUP and 16 G memory).

Magnetic resonance imaging of in vitro phantoms at 3 T. The phantom experiments were performed on a 3 T Bruker Biospec system (Bruker, Ettlingen, Germany). PCr phantoms with different concentrations (i.e., 10 ± 1 mM, 20 ± 1 mM, 40 ± 1 mM, 60 ± 1 mM, and 80 ± 1 mM) were prepared. Phantoms were prepared in phosphate-buffered saline (PBS) titrated to pH 7.3 $\pm$ 0.1. All samples were studied in 10 mm glass tubes. A continuous wave saturation module with a saturation power of 0.6 μT and a saturation time of 10 s was applied. Data were acquired using a turbo spin echo (TSE) sequence with $\text{TR/TE} = 13 \text{ s}/4.5 \text{ ms}$, TSE factor = 32, slice thickness of 2 mm, acquisition matrix size of 32×32 . Zero filling was applied for the Fourier transform and lead to a reconstructed image with a matrix size of 64×64 , and a resolution of $0.47 \times 0.47 \text{ mm}^2$. The CEST experiments

were carried out with only first-order B_0 shimming. The B_0 map was obtained via the WASSR method¹⁶, in which 21 offsets from -0.5 to 0.5 ppm were collected with acquisition parameters and geometry identical to other CEST experiments. The saturation offsets were swept from 0.5 to 4 ppm with an increment of 0.1 ppm. A 0.05 ppm increment was used between 1.5 ppm and 3 ppm to facilitate the detection of CEST peak. The image collected with an offset of 100 ppm was used as the S_0 image. The T_1 and T_2 maps were also collected on the phantom and were applied for the generation of training data. T_1 maps were acquired using an inversion recovery sequence with inversion time (TI) from 7 ms to 11.5 s and $TR = 15$ s, while the T_2 map was obtained by a Carr–Purcell–Meiboom–Gill (CPMG) echo train followed by a TSE readout⁴⁷ with geometry identical to that of the T_1 map measurement. In both T_1 and T_2 measurements, a slice thickness of 4 mm and a matrix size of 32×32 were applied. In the T_2 CPMG experiment, the inter-echo time was fixed at 5 ms and the total echo time was varied by increasing the number of echoes (40), while block pulses were used for the 90° excitation, and 90° – 180° – 90° composited pulses were applied for the 180° refocusing. T_1 values were found to be 2.6 ± 0.02 s for all PCr phantoms, while T_2 values were found to be 1.8 ± 0.05 s.

Magnetic resonance imaging on human skeletal muscle at 3 T. The Johns Hopkins Institutional Review Board approved all human studies, with trial registration at ClinicalTrials.gov (NCT04234880). The outcomes of clinical trial are high-resolution PCr and creatine mapping of human skeletal muscle. The inclusion criteria are (1) Subject must be at least 18 years of age. (2) Subject must be willing and able to undergo verbal and written informed consent. (3) Healthy subjects will have no history of cardiovascular or peripheral vascular disease, diabetes, claudication, or difficulty walking. The exclusion criteria are (1) Unable to understand the risks, benefits, and alternatives of participation and give meaningful consent. (2) Contraindications to MRI scan (e.g., implanted metallic objects). (3) Significant cardiovascular (heart failure, significant coronary artery disease, infiltrative or hypertrophic cardiomyopathy, constrictive pericarditis), pulmonary or musculoskeletal, or orthopedic disease that significantly limit exercise capacity. (4) Weight > 350 lbs (inability to fit in the MRI). (5) Cognitive or speech impairments that would limit completion of questionnaires or fatigue reporting. (6) Subjects with rest pain, critical limb ischemia will be excluded for the study. (7) Pregnant women. All subjects gave informed written consent after explanation of the study and protocol. All procedures involving human participants were in accordance with the ethical standards of the institutional and national ethical regulations. The optimization of PCr CEST signal and PCr mapping on resting-state skeletal muscle were performed on a 3 T Philips MRI system equipped with a 16-channel knee coil for radio-frequency transmission and reception. Seven healthy subjects (age 27 ± 5 years) were recruited for the PCr optimization and high-quality PCr mapping. The FOV for human skeletal muscle experiment was 160×160 mm². High-resolution T_2 weighted images were collected for anatomical referencing using TSE sequence with a resolution of $0.63 \times 0.63 \times 5.0$ mm³. A continuous wave saturation module with a saturation power of 0.6 μ T and a saturation time of 800 ms was applied for PCr mapping on resting-state human skeletal muscle. Images were acquired using a single-shot TSE sequence with $TR = 3.5$ s, $TE = 3.7$ ms, TSE factor = 37 , and a resolution of $2.2 \times 2.2 \times 5.0$ mm³. The Z-spectra between 1.3 ppm and 3.5 ppm were sampled together with two S_0 images collected at an offset of 100 ppm. Number of offsets acquired for the Z-spectrum was 52 and total scan time was 3 min. B_0 maps were obtained using a dual-echo sequence with $TR = 10$ ms, $TE = 4.6$ ms, and flip angle of 30° . B_1 maps were obtained using the dual refocusing echo acquisition mode (DREAM) technique with a stimulated echo acquisition mode (STEAM) flip angle of 60° ²³. When optimizing the PCr signal in muscle, the extraction of the PCr signal was achieved by the PLOF method^{17,18}. The Z-spectrum between 1.6 and 3.5 ppm was utilized for the two-step fitting in the PLOF method, and two regions of the Z-spectrum, i.e., 1.6 – 2.1 ppm and 2.9 – 3.5 ppm, were selected for the background fitting. A single Lorentzian function was assumed for the PCr CEST peak at 2.5 ppm. The observed PCr CEST signal ΔZ_{PCr} was given by taking the difference between the fitted background (Z_{back}^{fit}) and the observed Z value at the 2.5 ppm, i.e., ($Z_{back} - Z_{2.5}$).

The CEST experiments and ^{31}P 2D MRS on exercised human skeletal muscle were performed on a second 3 T Philips MRI system equipped with a 2-channel surface coil for ^1H imaging and a ^{31}P excite/receive coil placed beneath the calf muscle for ^{31}P 2D MRS. Four healthy subjects (age 27 ± 5 years) were recruited for in-magnet plantar flexion exercise. The exercise protocol involved plantar flexion exercise with repetitively lifting a 16 lb weight at a rate of 1 Hz for 80 s, and then holding the load for 90 s before stopping all exercise to allow subsequent measures of postexercise PCr recovery⁴⁸. CEST images were acquired using a single-shot TSE sequence with $TR = 3$ s, $TE = 9.3$ ms (the shortest TE available), TSE factor = 37 , and a resolution of $2.2 \times 2.2 \times 20.0$ mm³. The Z-spectra between 1.3 ppm and 3.5 ppm were sampled together with one S_0 image collected at an offset of 100 ppm. Number of offsets acquired for the Z-spectrum was 30 and total scan time was 1.5 min. The ^{31}P 2D MRS was performed with $TR = 1.5$ s, $TE = 1.44$ ms, and a total scan time of 1.5 min. 60 measurements on an 8×8 k-space were acquired except for the four corners (circular k-space shutter). The k-space data were then reconstructed on a 16×16 grid leading to a voxel size of 10×10 mm². The slice thickness of ^{31}P 2D MRS was determined by the excitation profile of the ^{31}P coil, which is in the order of 80 mm.

Correlation analysis of PCr maps obtained by ANNCEST and ^{31}P 2D MRS. A correlation analysis was performed to compare PCr maps obtained by ANNCEST and gold-standard ^{31}P 2D MRS on resting and exercised human skeletal muscle. To remove the location mismatch in the CEST images caused by motion, image registration was applied to ANNCEST results using the Medical Imaging Registration Toolbox (MIRT). Because ^{31}P 2D MRS is less sensitive to motion with worse spatial resolution, the ANNCEST results were downsampled from 72×72 to 16×16 to match the matrix size of ^{31}P 2D MRS. The downsampling map was calculated by averaging the intensity within the corresponding patch on high-quality PCr mapping. The PCr maps of baseline, during holding, and 0.75 min of recovery were chosen for comparison. Because the ^{31}P was placed beneath the skeletal muscle and may lead to poor sensitivity to the upper-half FOV, only the regions close to the coil were chosen for correlation analysis. The pixel-by-pixel correlation analysis was accomplished using Matlab built-in function “corrcoef”.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The source data underlying Figs. 1d–n, 2c–g, 3a–i, 4b–h, 5b–g are provided as a Source Data file. The other data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

The code used in this study is provided in Supplementary Data 1. We also deposit the code in <https://github.com/LinChenMRI/ANNCEST.git>.

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Author contributions

L.C. and J.X. designed and performed experiments, processed data, and wrote the paper; P.v.Z. and R.W. helped with designing the study, interpreting the data, and paper editing; R.W. and M.S. helped with the ³¹P 2D MRS experiments and processing. K.C. and J.H. prepared the phantoms and performed the phantom MRI experiments; M.S. and Q.Q. provided pulse sequence design and technical guidance on the human subject scanning. H.L. and Z.W. helped with human experiments.

Competing interests

The authors declare no competing interests.

Additional information

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NOTE

High-sensitivity CEST mapping using a spatiotemporal correlation-enhanced method

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Purpose: To obtain high-sensitivity CEST maps by exploiting the spatiotemporal correlation between CEST images.

Methods: A postprocessing method accomplished by multilinear singular value decomposition (MLSVD) was used to enhance the CEST SNR by exploiting the correlation between the Z-spectrum for each voxel and the low-rank property of the overall CEST data. The performance of this method was evaluated using CrCEST in ischemic mouse brain at 11.7 tesla. Then, MLSVD CEST was applied to obtain Cr, amide, and amine CEST maps of the ischemic mouse brain to demonstrate its general applications.

Results: Complex-valued Gaussian noise was added to CEST k-space data to mimic a low SNR situation. MLSVD CEST analysis was able to suppress the noise, recover the degraded CEST peak, and provide better CrCEST quality compared to the smoothing and singular value decomposition (SVD)-based denoising methods. High-resolution Cr, amide, and amine CEST maps of an ischemic stroke using MLSVD CEST suggest that CrCEST is also a sensitive pH mapping method, and a wide range of pH changes can be detected by combining CrCEST with amine CEST at high magnetic fields.

Conclusion: MLSVD CEST provides a simple and efficient way to improve the SNR of CEST images.

KEYWORDS

amide, amine, chemical exchange saturation transfer (CEST), creatine (Cr), ischemic stroke, pH mapping, polynomial and Lorentzian line-shape fitting (PLOF), multilinear singular value decomposition (MLSVD), singular value decomposition (SVD)

1 | INTRODUCTION

CEST MRI is a versatile technique that can significantly enhance the sensitivity of detecting low concentrations of proteins and metabolites through their exchangeable protons.¹⁻⁸ Nowadays, this technique has been successfully applied

to detecting proteins⁹⁻¹¹ and nuclear overhauser enhancement¹²⁻¹⁶ in tissues, as well as various endogenous metabolites such as glycogen,¹⁷ glutamate,¹⁸ glycosaminoglycan,¹² creatine,¹⁹⁻²³ and phosphocreatine.^{19,24-26} However, due to the strong scaled-down effect produced by the magnetization transfer contrast induced by the macromolecules, CEST

contrasts in tissues are significantly reduced compared to the CEST signal in solutions,²⁷⁻³⁰ which leads to a diminished SNR and challenges the accuracy of CEST quantification. Many efforts have been devoted to optimizing certain saturation parameters (e.g., saturation power and length) to increase CEST contrasts in tissues,³⁰⁻³³ but improvements following this strategy have been limited. Reducing the noise and oscillations in the Z-spectrum is an alternative way to improve the CEST SNR. For example, some of the noise and oscillations in the Z-spectrum are introduced through subject physiological motions or MRI instability, which could be solved using motion-insensitive MRI methods, such as the self-gated technique UTE-CEST,¹³ or using navigators to correct frequency fluctuations.^{34,35} Some postprocessing methods have also been proposed to reduce the noise and oscillations in the Z-spectrum by exploiting the redundancy of CEST images between different saturation offsets, such as singular value decomposition (SVD)^{36,37} and principal component analysis.³⁸⁻⁴⁰

Recently, a powerful reconstruction method, called *spectroscopic imaging by exploiting spatio-spectral correlation*,^{41,42} and a postprocessing tensor factorization denoising method⁴³ have been developed in the MRS community by making use of a property known as the partial separability of spectroscopic signals. This property indicates that high-dimensional spectroscopic signals can be well represented by a very low-dimensional subspace, and that the SNR of MRS can be dramatically improved without sacrificing accuracy. Noted that this partial separability property is also exploited in SVD and principal component analysis methods implemented by 2D tensor decomposition.³⁶⁻⁴⁰ Inspired by these techniques, we here proposed a higher-order tensor decomposition for better exploiting the partial separability of CEST images and improving the SNR of CEST contrasts. The higher-order tensor decomposition was accomplished using multilinear singular value decomposition (MLSVD).⁴⁴ The performance of the MLSVD CEST was compared with that of other denoising methods in CEST MRI. For the application and validation of the method, MLSVD CEST

was applied to obtain high-resolution Cr, amide, and amine CEST images from mouse brains using an ischemic stroke model. The wide range of Cr, amide, and amine CEST intensities, due to the pH drop in the infarct and penumbra zones,^{11,45} forms an excellent model to test the efficiency and accuracy of MLSVD CEST.

2 | METHODS

2.1 | MLSVD CEST theory

The purpose of MLSVD CEST is to remove noise in the Z-spectrum using a tensor decomposition-based low-rank denoising method. Without losing generality, the following derivation assumes the acquisition of a series of 2D images with different frequency offsets, whereas the processing of a series of 3D images with different frequency offsets can be performed in complete accordance with the procedures hereafter.

For 2D images with different frequency offsets, the acquired CEST data can be stored in a 3D array matrix $\mathbf{M}$ with dimensions x , y , and n . The variables x and y refer to the number of pixels in the 2 spatial domains, and n stands for the number of frequency offsets adopted in the Z-spectrum (Figure 1B). The acquired data $\mathbf{M}$ can be written in the following form using MLSVD⁴⁴:

$$\mathbf{M}^{x \cdot y \cdot n} = \mathbf{S}^{x \cdot y \cdot n} \cdot \mathbf{U}_1^{x \cdot x} \cdot \mathbf{U}_1^{y \cdot y} \cdot \mathbf{U}_3^{n \cdot n}, \quad (1)$$

where $\mathbf{U}_1$, $\mathbf{U}_2$, and $\mathbf{U}_3$ are orthonormal matrices, and $\mathbf{S}$ refers to the core tensor. MLSVD was accomplished by the Tensorlab v3.0 toolbox (<https://www.tensorlab.net/>). Due to the spatio-spectral correlation of CEST data, $\mathbf{M}$ can be approximately represented by a low-rank model with reduced noise level.^{41,42,46}

The rank numbers x_{trun} , y_{trun} , and n_{trun} were adopted to truncate the orthonormal matrices and core tensor, and the denoising CEST data could then be obtained using the following equation:

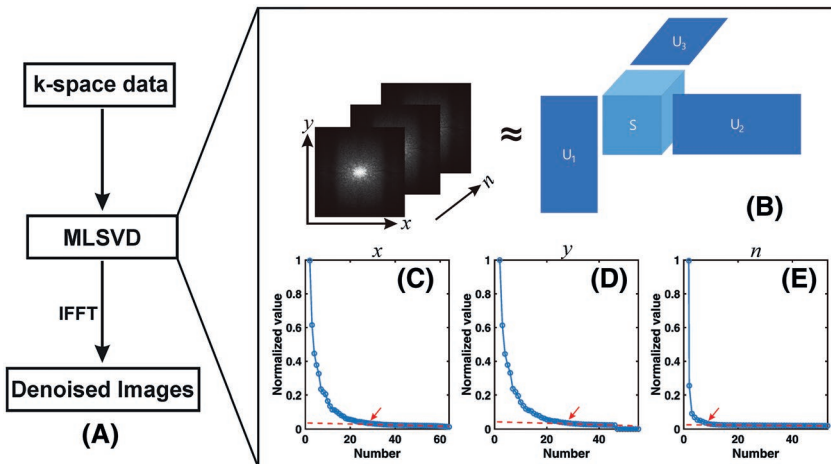


FIGURE 1 (A) Flowchart of MLSVD denoising. (B) Schematic diagram of truncated MLSVD of k-space data at different frequency offsets n . (C~E) The multilinear singular values along different dimensions (2 spatial, 1 frequency offset). The rank number at the corner indicated by the red arrow was selected to truncate the orthonormal matrices and core tensor. MLSVD, multilinear singular value decomposition

$$M_{denoise}^{x \cdot y \cdot n} = S^{x_{trun} \cdot y_{trun} \cdot n_{trun}} \cdot U_1^{x \cdot x_{trun}} \cdot U_2^{y \cdot y_{trun}} \cdot U_3^{n \cdot n_{trun}}. \quad (2)$$

The truncated rank numbers were set to the corner of multilinear singular value L-curve along different dimensions using the following steps. First, the last part (40% in this study) of the plot of multilinear singular value versus rank number was selected to fit a linear reference line. Second, the multilinear singular values were subtracted from this linear reference line, and the first index for which the difference was smaller than a certain threshold (0.02 in this study) was selected as the truncated rank number.

2.2 | Animal preparation

The procedures on mice were approved by the Johns Hopkins University Animal Care and Use Committee. The transient middle cerebral artery occlusion stroke model was performed on 3 C57BL/6 male mice (age: 5 months). The mice were anesthetized initially with 4% isoflurane followed by 2% isoflurane during surgery. An incision was made at the midline of the neck, and the common carotid artery and external carotid artery were ligated with silk suture. A filament was inserted into the external carotid artery and threaded forward into the internal carotid artery until the tip occluded the origin of the middle cerebral artery. An middle cerebral artery occlusion period of 60 min was applied, followed by reperfusion. The MRI experiments were performed approximately 1 h after surgery.

2.3 | MRI experiments

All MRI experiments were performed on a horizontal bore 11.7 T (tesla) Bruker Biospec system (Bruker, Ettlingen, Germany). For the animal studies, a 72-mm quadrature volume resonator was used as a transmitter, and a 4-element (2 × 2) phased array cryoprobe was used as a receiver. The animals were anesthetized using 2% isoflurane in medical air, followed by 1% to 1.5% isoflurane for maintenance during the MRI scan. The mouse head was positioned using a bite bar and 2 ear pins. During the MRI scan, the mice were placed on a water-heated animal bed equipped with temperature and respiratory controls. Respiratory rates were monitored via a pressure sensor (SAII, Stony Brook, NY) and maintained at 30 to 50 breaths per min. The B_0 field over the image slice was adjusted using field-mapping and second-order shimming.

All CEST experiments were recorded using continuous-wave CEST experiments. MR images were acquired using a turbo spin echo sequence with TE = 18 ms, TR = 5 s, turbo spin echo factor = 20, slice thickness = 1 mm, matrix size of 64 × 64, and resolution of 0.25 × 0.25 mm². Different saturation powers and saturation lengths were used for Cr, amine, and amide CEST MRIs

(i.e., 2 μT and 1 s for Cr, 3 μT and 0.4 s for amine, 1 μT and 3 s for amide), because previous studies had already demonstrated that the maximum amide CEST is achieved with 1 μT saturation power in a high field.⁴⁷ Although the maximum CrCEST guanidinium signal occurs at around 1.5 μT,³⁰ 2 μT was chosen to minimize the competing protein guanidinium signal.²² Similar to CrCEST, the maximum amine CEST was achieved with 1.5 to 2 μT saturation power³⁰; however, a high saturation power (3 μT) was also used for the amine CEST to suppress contaminations from slow or intermediate exchanging protons.³⁰ The saturation lengths were chosen based on the discovery that the optimal saturation length for in vivo CEST signals is determined by $R_{1\rho}$ relaxation time.³⁰ The R_1 relaxation of the mouse brain was measured with rapid acquisition with relaxation enhancement sequence with variable TR (TR = 5.5, 3.0, 1.5, 0.8, 0.5, 0.3 s), with image slices identical to those used in the CEST studies.

2.4 | CEST mapping using PLOF

The polynomial and Lorentzian line-shape fitting (PLOF) method for extracting a CEST signal with a discernible peak previously has been well described.^{22,24,26,30} Briefly, the normalized saturation signal at each frequency offset is given by⁴⁸⁻⁵¹:

$$Z(R_{1\rho}) = (1 - Z^{ss}) e^{-R_{1\rho} t_{sat}} + Z^{ss} \quad (3)$$

$$Z^{ss} = \frac{\cos^2 \theta R_1}{R_{1\rho}}, \quad (4)$$

where Z^{ss} is the steady-state Z-spectral intensity; R_1 is the longitudinal relaxation rate of water; t_{sat} is the saturation time; and $\theta = \tan^{-1} \omega_1 / \Delta$ is the tilt angle of the effective magnetization with respect to the Z-axis induced by a saturation pulse with nutation frequency ω_1 at frequency offset Δ . $R_{1\rho}$ is the water relaxation rate under the saturation pulse, which includes contributions from the effective water relaxation R_{eff} and an apparent relaxation term R_{back} related to all saturation transfer processes other than the detected CEST signal (R_{exch}) in tissue⁵⁰:

$$R_{1\rho} = R_{eff} + R_{back} + R_{exch}, \quad (5)$$

where $R_{eff} = \cos^2 \theta R_1 + \sin^2 \theta R_2$ is the measured longitudinal relaxation rate of water in the rotating frame without additional solution components. The observed CEST signal ΔZ is given by:

$$\Delta Z = Z(R_{eff} + R_{back}) - Z(R_{eff} + R_{back} + R_{exch}). \quad (6)$$

The CEST peak (R_{exch}) and broad background signal (R_{back}) in Z-spectrum can be represented as a Lorentzian function and a polynomial function, respectively:

$$R_{exch} = R_{exch}^{max} \frac{(w/2)^2}{(w/2)^2 + (\Delta - \Delta_{exch})^2} \quad (7)$$

$$R_{back} = \sum_{n=1}^M C_n (\Delta - \Delta_{exch})^n, \quad (8)$$

where w is the peak FWHM of the Lorentzian line-shape. R_{exch}^{max} is the true apparent relaxation rate contribution of the CEST peak. Δ_{exch} is the chemical shift offset of the CEST peak relative to the water signal. C_n terms are the 0 to M -order polynomial coefficients. The initial chemical shift offsets δ_{ini} for Cr, amide, and amine were set to 1.95 ppm, 3.6 ppm, and 2.7 ppm with respect to water, respectively. The Z-spectral range of $[\delta_{ini} - 1, \delta_{ini} + 1]$ (ppm) was used for PLOF. For Cr and amide, the Z-spectral range between $[\delta_{ini} - 0.4, \delta_{ini} + 0.4]$ (ppm) was excluded from the background fitting. For amine, the Z-spectral range between $[\delta_{ini} - 0.6, \delta_{ini} + 0.6]$ (ppm) was excluded from the background fitting, which was determined by the optimization shown in Supporting Information Figure S3.

3 | RESULTS

3.1 | Validation of MLSVD CEST

A flowchart of MLSVD denoising is shown in Figure 1, including a schematic diagram of MLSVD CEST in Figure 1B.

The original CrCEST maps were used as a reference, and complex-valued Gaussian noise was added to CEST k-space data to mimic a low SNR situation. In MLSVD CEST, the noise of the CEST data was suppressed by truncating the orthonormal matrices and core signal intensity tensor. As shown in Figure 1B through 1D, the multilinear singular values decayed dramatically with the increase of rank number, especially along the offset dimension. The rank numbers around the corner of the L-curve, indicated by the red arrow, were chosen for truncation, which were 30, 30, and 10, for x , y , and n dimensions, respectively. From the difference maps shown in Supporting Information Figure S1, it can be seen that the adopted truncated rank numbers could efficiently remove noise while maintaining useful information such as image contrasts.

The original Z-spectrum from a small region of interest before noise addition is plotted in Figure 2A for reference. The Z-spectrum with noise added is plotted in Figure 2C. The Z-spectra obtained by the smooth method via the MatLab (Mathworks, Natick, MA, version: 9.8.0.1396136 (R2020a) Update 3) build-in function *smooth* with a span number of 3 and the SVD method⁴⁰ from the same CEST images with added noise are illustrated in Figure 2D,E, respectively. The choice of truncated rank number for the SVD method is illustrated in Supporting Information Figure S2. These extracted Z-spectra were fitted using the PLOF method following Equations (6)-(8). Without additional denoising, the CEST peak at 1.95 ppm was indiscernible and could not be well fitted with PLOF, as shown in Figure 2C. From Figure 2D,E,

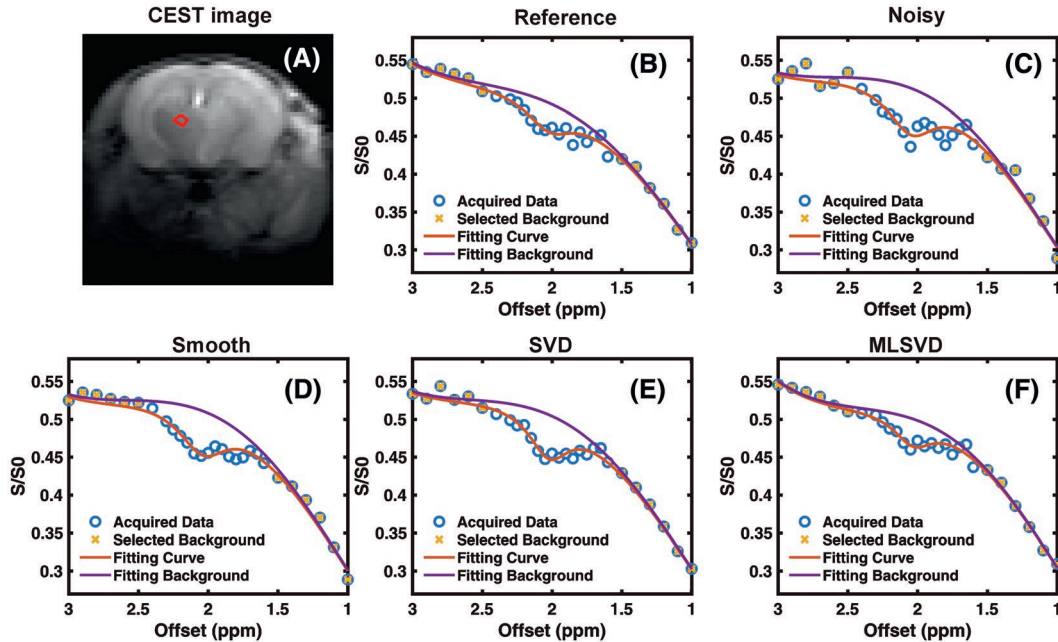


FIGURE 2 Effect of different denoising methods on the Z-spectrum. The noisy spectra were obtained from image data after adding complex-valued Gaussian noise to CEST k-space data. (A) M_0 image at $\Delta\omega = 200$ ppm. The ROI circumscribed by the red circle was selected for extracting a Z-spectrum. (B) Reference Z-spectrum before adding noise. (C) Z-spectrum extracted from noisy images without denoising. Z-spectrum obtained after applying (D) smooth function, (E) SVD, and (F) MLSVD. ROI, region of interest; SVD, singular value decomposition

it can be seen that both smooth and SVD methods can reduce the oscillation induced by additional noise. However, the restored CEST contrast (5.56% for smooth and 5.82% for SVD) was clearly larger than the original before noise addition (3.83%). The results in Figure 2F show that MLSVD CEST can well suppress the noise and properly recover the degraded CEST peak. The CEST contrast obtained by MLSVD CEST (3.66 %) was closest to the gold standard original.

To further evaluate the performance of different denoising methods, CrCEST maps (e.g., the R_{exch}^{max} (1.95 ppm) maps) were calculated and displayed in Figure 3. Due to the lower pH values in the ischemic regions, a clearly reduced Cr CEST contrast was observed on the ipsilateral side of the mouse brain. However, it is more difficult to distinguish the ischemic regions in the CrCEST with added noise, as shown in Figure 3C. All 3 of these methods yielded much improved CrCEST maps compared to that without denoising, in which the ischemic region could be well distinguished. To provide an objective evaluation, we calculated the voxel-by-voxel linear regression between the denoising results and the

reference. The Bland-Altman plots between the reference and the denoising results are also given in Figure 3G through 3-I. From the linear regression scores and Bland-Altman plots, it is clear that MLSVD CEST yielded the best CrCEST map among the 3 denoising methods.

3.2 | Cr, amide, and amine maps with MLSVD CEST

We further applied MLSVD CEST to obtain Cr, amide, and amine CEST maps in vivo in the mouse middle cerebral artery occlusion stroke model; the results are plotted in Figure 4. Overall, the image quality improved significantly after MLSVD processing (Figure 4A,B,E,F,I,J). The ischemic regions are more clearly visible after MLSVD for all proton types. Because both Cr and amide CEST show distinguishable peaks in the Z-spectra at physiological pH values, as shown in Figure 4D and H, respectively, their CEST signals could be extracted via PLOF, and the maps

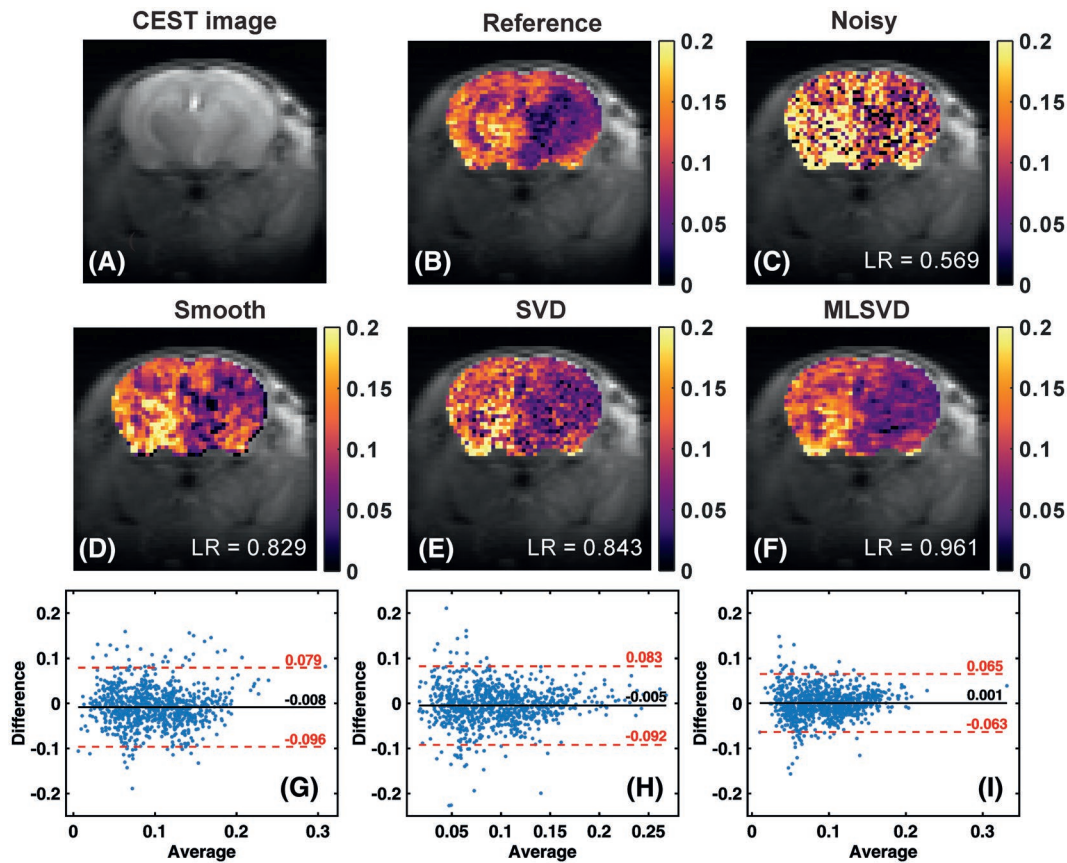


FIGURE 3 Cr R_{exch}^{max} maps obtained by PLOF with different denoising methods. (A) M_0 image at $\Delta\omega = 200$ ppm. (B) Reference (gold standard) R_{exch}^{max} map without noise added. (C) R_{exch}^{max} map determined from Z-spectra with Gaussian noise imposed in k-space. The denoising R_{exch}^{max} maps obtained by (D) the smooth function, (E) SVD method, and (F) MLSVD approach before applying PLOF. The LR between reference and denoising map was calculated by MatLab (MathWorks, Natick, MA, version: 9.8.0.1396136 (R2020a) Update 3) built-in function *regress*. Bland-Altman plots between the reference and the denoising results obtained by (G) the smooth function, (H) SVD, and (I) MLSVD, respectively. LR, linear regression; PLOF, polynomial and Lorentzian line-shape fitting

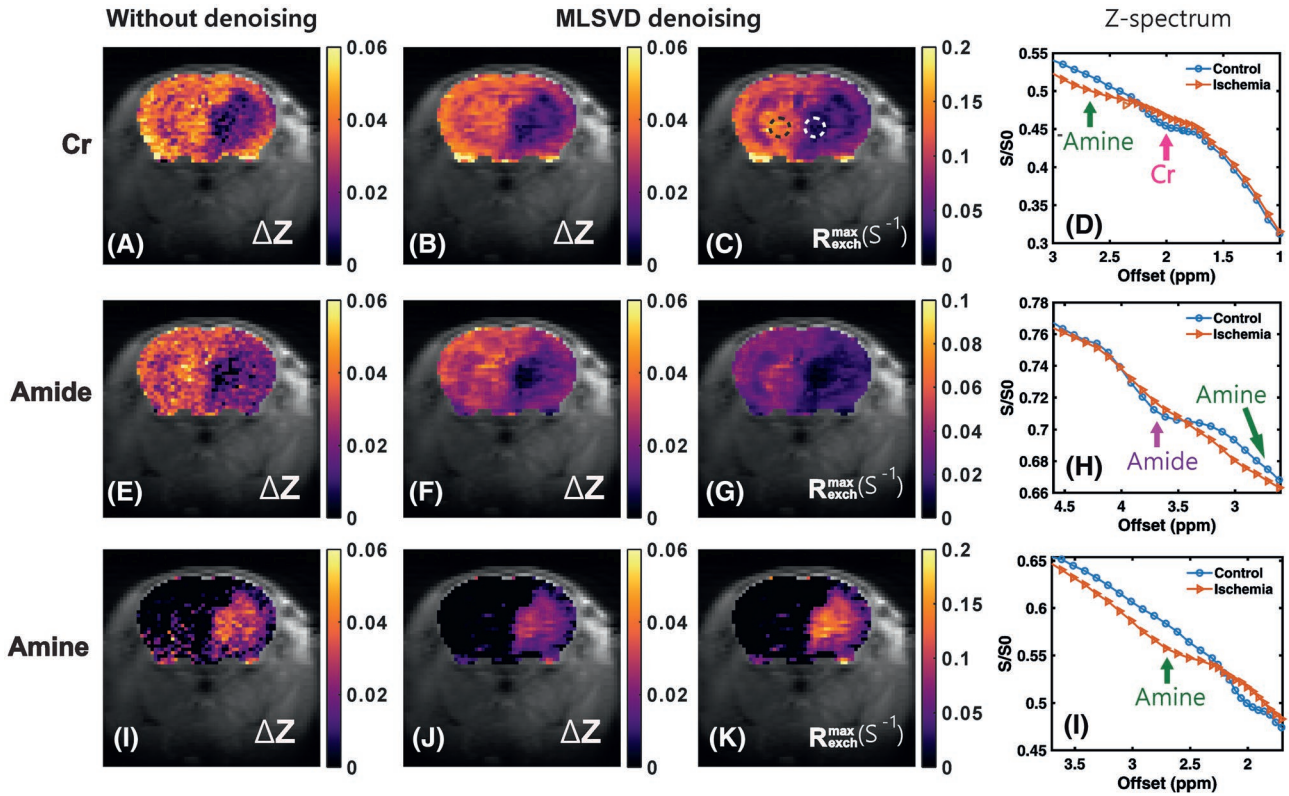


FIGURE 4 Typical ΔZ CEST maps obtained from the PLOF method for Cr (A,B), amide (E,F), and amine (I,J) protons. CEST maps obtained before (A,E,I) and after (B,F,J) applying the MLSVD method. The MLSVD CEST $R_{\text{exch}}^{\text{max}}$ maps for Cr (C), amide (G), and amine (K) are shown together with the Z-spectra extracted from the main ischemic region (white dashed circle in (C)) and a contralateral control region (black dash circle in (C)) for Cr (2 μT and 1s) (D), amide (1 μT and 3s) (H), and amine (3 μT and 0.4s) (I), respectively. T, tesla

are plotted in Figure 4A-C,E-G. Both the Cr and amide contrasts show strong pH dependence, as indicated by the much-reduced Cr and slightly-reduced amide CEST values in the ischemic zone and confirmed by the Z-spectra in the main ischemic region in which both Cr and amide CEST peaks are barely observable (Figure 4D,H). The CrCEST effect ($\Delta Z = 0.036 \pm 0.05$) was slightly higher than the amide CEST ($\Delta Z = 0.03 \pm 0.06$) peak for this MRI field and saturation power, as shown by the ΔZ values in the contralateral regions (Figure 4B,F). For the amine CEST, there was no resonance visible in the spectrum at physiological pH (Figure 4L). However, a peak could be clearly seen at lower pH⁵² that could be extracted via the PLOF method. The amine-weighted image is significantly enhanced at low pH values (Figure 4I-K), which is opposite to the Cr and amide CEST contrasts, indicating that the slowed-down exchange places the proton exchange rate in a more appropriate exchange regime for CEST detection.

4 | DISCUSSION

In this paper, we tested *MLSVD CEST* to improve the SNR of the Z-spectrum and yield high-sensitivity CEST maps of mouse brains. By selecting appropriate truncation numbers

in the spatial and offset domains, we demonstrated that the MLSVD denoising algorithm could reduce noise significantly while maintaining CEST contrasts. This is critical for CEST applications, considering that the majority of them have a magnitude on the order of only a few percents of the water signal.

One-dimensional denoising methods, such as the smooth function, remove noise voxel-by-voxel by averaging the Z-spectrum over certain range; however, for Z-spectra with low CEST contrast and high noise levels, such methods will suppress both noise and CEST contrast, leading to greatly underestimated quantification results (Figures 2D and 3D). SVD and principal component analysis methods utilize the spectral correlation between all voxels throughout the entire image to reduce noise levels.³⁶⁻⁴⁰ Compared to SVD and principal component analysis methods, MLSVD CEST can exploit the additional spectral correlation between all the rows and columns of the CEST images and hence yield better noise reduction performance, as demonstrated in Figures 2 and 3. MLSVD and SVD retain optimal performance when the noise distribution in complex k-space is Gaussian. Note that MLSVD and SVD can also be applied to the magnitude image domain when the raw data or complex image space data is unavailable. In that case, the performance of MLSVD and SVD may be further improved by adapting the noise

model to fit magnitude Rician noise,⁵³ as previously demonstrated by Lam et al.⁵⁴

MLSVD CEST can be applied to other CEST contrasts, as demonstrated in the amide and amine CEST images (Figure 4). The CEST signal at 1.95 ppm is a mixture of slow-exchanging protein and intermediate-exchanging Cr guanidinium protons, as recently shown in a knockout mouse model²² and an ex vivo study using homogenous rat brain tissue.²³ Previous studies have demonstrated that the 1.95 ppm signal is dominated by guanidinium protons²² with low saturation powers ($<0.6 \mu\text{T}$), and the exchange rate increases with lower pH values across the physical pH range.^{55,56} A similar pH dependence was observed for urea.⁵⁷ On the other hand, the exchange rate of the Cr guanidinium protons decreased significantly with lower pH values, as confirmed by phantom studies.⁵⁸ The current pH-dependent CrCEST results confirm our previous result that, with a saturation power of $2 \mu\text{T}$, Cr dominates the CEST signal at 1.95 ppm with a contribution of around 66%.³⁰ Our data also indicate that CrCEST can be applied to mapping pH in the brain at high fields, similar to amide CEST. However, one needs to be cautious in the interpretation because the Cr concentration will increase in ischemic regions due to the conversion of phosphocreatine into Cr.^{59,60} Under the current acquisition conditions, we were also able to observe the amine peaks from mobile proteins at lower pH values, with a center frequency at around 2.7 ppm. The amine CEST showed potential for sensitive pH mapping in low pH ranges because the amine CEST increases strongly; however, in this case it is due to the much-reduced exchange rates at the low pH values, leading to better detection by moving from the fast- to intermediate-exchange regime. Recently, amine-based CEST mapping²⁹ has also been suggested for tumor pH mapping.^{61,62}

The measurement of T_2 is not necessary for the calculation of R_{eff} due to the high magnetic field applied in this study, which leads to large frequency offsets. The saturation powers used for the Cr (at offset 1.95 ppm), amine (at 2.7 ppm), and amide (at 3.6 ppm) CEST experiments were $2 \mu\text{T}$, $3 \mu\text{T}$, and $1 \mu\text{T}$, respectively. According to the equation $\theta = \tan^{-1} \omega_1 / \Delta$, this corresponds to $\sin^2 \theta$ terms for Cr, amine, and amide CEST of 0.0076, 0.0089, and 0.0006, respectively. Therefore, the R_{eff} term can be simplified as $R_{\text{eff}} = \cos^2 \theta R_1 + \sin^2 \theta R_2 \approx R_1$ in this study. However, the T_2 measurement may become important at lower MRI fields for which the $\sin^2 \theta$ term can no longer be neglected.

5 | CONCLUSION

MLSVD CEST was developed to provide a simple and efficient way to improve the SNR of CEST images. The method outperformed conventional smoothing and SVD methods. High-resolution Cr, amide, and amine MLSVD CEST maps could be acquired on a mouse model of ischemic stroke,

demonstrating that CrCEST, amide CEST, and amine CEST are sensitive pH mapping methods at 11.7T. We expect that maps for a wide range of pH values can be achieved by combining CrCEST and amine CEST at high magnetic fields.

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DATA AVAILABILITY STATEMENT

The code that supports the findings of this study is openly available at <https://github.com/LinChenMRI/MLSVD-CEST.git>.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 (A) Noisy CEST images. (B) Denoised CEST images using MLSVD. (C) The difference map between noisy and denoised images

FIGURE S2 The singular value distribution obtained by SVD method. The truncated rank number was set to 10

FIGURE S3 (A) ΔZ of amine CEST peak obtained by PLOF as a function of different peak ranges. (B) Representative PLOF result for amine CEST signal with a peak range of 1.2 ppm

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