

Ultra-low field ^{13}C MRI of hyperpolarized pyruvate

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This is a strong manuscript introducing a notable advance in low-field hyperpolarized ^{13}C MRI. The experimental methodology is rigorous, the claims are well supported, and the impact on the field is likely substantial. Addressing the points below would further strengthen the paper.

- The reported ~3.3% ^{13}C polarization is derived from comparison with the thermal acetic acid spectrum shown in Figure 1, which exhibits very low SNR and resolves only two peaks of the expected quartet. If this spectrum is indeed the reference used to compute the polarization, the resulting value must carry a substantial uncertainty. Although the fitting procedure presented in the Supplementary Material demonstrates careful attention to this limitation, using a more concentrated ^{13}C -labeled compound, or a thermal reference acquired with a significantly higher number of averages, would provide a much more robust and reliable calibration of the thermal polarization.

- T_1 and T_2 measurements are performed under continuous pH_2 bubbling (Supplementary Figures 4 and 6). The ongoing bubbling during the acquisitions may introduce convection, susceptibility fluctuations, and residual SABRE-related polarization effects, potentially influencing the apparent mono-exponential decay, as is clearly visible in Supplementary Figure 6. The manuscript should explicitly discuss how these factors may bias the extracted relaxation times and clarify that the reported T_x values represent effective relaxation under continuous bubbling rather than intrinsic relaxation constants. Supplementary Fig. 6 suggests non-mono-exponential behavior in T_2 decay, warranting discussion of potential multi-component relaxation.

- The MRI data are presented exclusively in arbitrary/normalized units, without any absolute quantification of noise levels or SNR. In Figures 2 and 3, the intensity scales are normalized to the noise floor, preventing the reader from assessing the true performance of the imaging system and the practical detectability of hyperpolarized ^{13}C at 6.5 mT. The manuscript should report quantitative SNR values (e.g. peak signal/standard deviation of noise in a signal-free region) or CNR values and, ideally, sensitivity expressed per μmol of ^{13}C . These metrics are essential for meaningful comparison to existing hyperpolarization and low-field MRI technologies and for evaluating how close the current implementation is to in vivo-relevant performance.

- The discussion section does not fully address challenges such as catalyst removal, purification, achievable bolus volumes, transfer times, and the impact of shorter T_1 at low field. A more balanced assessment of the remaining hurdles for translation would provide important context for the reader.

Reviewer #2

(Remarks to the Author)

The manuscript by Boele et al, presents technically impressive work demonstrating hyperpolarized ^{13}C MRI at ultra-low field and resolving chemical shift features of pyruvate and its metabolites. The imaging and spectroscopy results, particularly the ability to perform balanced steady-state readouts and distinguish spectral features at 6.5 mT, represent a meaningful contribution. The study is clearly relevant to ongoing efforts aimed at reducing costs and increasing accessibility in metabolic MRI.

However, several conceptual points require clarification, and the narrative would benefit from realignment. In its current form, the manuscript emphasizes aspects that are not central to the actual novelty, while providing insufficient clarity on the broader translational context and on what this work does (and does not) address.

Major comments:

1. In the abstract and introduction, the authors state that the limited use of hyperpolarized ^{13}C MRI in the clinic is “primarily due to a lack of access to affordable technology.” This is not accurate and misrepresents the current state of clinical translation. Dynamic nuclear polarization equipment is indeed expensive, but cost has not been the principal barrier to adoption. It is the lack of evidence demonstrating that hyperpolarized ^{13}C measurements improve diagnosis or guide treatment decisions that is missing. The field is still establishing clear, validated clinical indications. Cost becomes a secondary factor once a clinical indication is established, as the authors themselves have previously demonstrated with ultra-low-field proton MRI. Please reframe the rationale to reflect that the main challenge is the absence of fully established clinical use-cases; cost reduction is valuable but not the determining obstacle.
2. Much of the manuscript focuses on the specifics of SLIC-SABRE polarization. While SLIC-SABRE is the chosen method for creating the hyperpolarized ^{13}C pyruvate enhancement, the main technical novelty of the present work is ultra-low-field imaging and spectral discrimination, not the polarization mechanism. Clarify explicitly why SLIC-SABRE is discussed at this level of detail, if there is a specific scientific justification, it should be stated; if not, significantly shorten the detailed information of SLIC-SABRE. A short paragraph acknowledging the method's cost-effectiveness and compatibility with low fields should suffice.

Minor:

1. Add a short paragraph acknowledging that a clinical indication for ^{13}C pyruvate MRI at low field remains to be established and while cost-efficient hyperpolarization and ultra-low-field systems are valuable, validation of the clinical utility at high field is the decisive next step. You should cite the consensus for hyperpolarized ^{13}C for performing the neuroscience trials, that will pave the way for clinical utilization.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

I have no further questions. I appreciate the authors' careful revisions and their responsiveness to the reviewer comments. I recommend accepting this important work.

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Point-by-Point Response to Reviewers

Ultra-low field ^{13}C MRI of hyperpolarized pyruvate by Boele *et al.*

We thank the Reviewers for their thorough and constructive feedback. In revising the manuscript, we have re-framed the rationale to better reflect the barriers to clinical translation of hyperpolarized ^{13}C MRI, clarified experimental details relating to our measured ^{13}C polarization, T_1 , T_2 and MRI signal-to-noise, and further addressed the challenges specific to the development of SABRE polarization methods. These changes clarify the manuscript's positioning and better align the framing with the core technical contributions.

Reviewer 1

Reviewer #1 (Remarks to the Author):

This is a strong manuscript introducing a notable advance in low-field hyperpolarized ^{13}C MRI. The experimental methodology is rigorous, the claims are well supported, and the impact on the field is likely substantial. Addressing the points below would further strengthen the paper.

Authors' response: We thank the reviewer for praising the rigor of our methodology and the impact of our work. We are most grateful for their constructive criticism, which has strengthened our manuscript. A point-by-point response to each suggestion follows.

R1.1

The reported $\sim 3.3\%$ ^{13}C polarization is derived from comparison with the thermal acetic acid spectrum shown in Figure 1, which exhibits very low SNR and resolves only two peaks of the expected quartet. If this spectrum is indeed the reference used to compute the polarization, the resulting value must carry a substantial uncertainty. Although the fitting procedure presented in the Supplementary Material demonstrates careful attention to this limitation, using a more concentrated ^{13}C -labeled compound, or a thermal reference acquired with a significantly higher number of averages, would provide a much more robust and reliable calibration of the thermal polarization.

Authors' response: We agree that our estimation of ^{13}C polarization carries substantial uncertainty. To improve our confidence in our estimate we have repeated our thermal measurement in line with the reviewer's feedback. Increasing the number of averages from 256 to 1024, we averaged for 4 hours and 20 minutes on the same acetic acid sample. The resulting spectrum just has $2\times$ that SNR, allowing us to better fit the expected quartet. The resulting integral provides a ^{13}C polarization estimate of $\sim 3.45\%$, agreeing within 5% of our original estimate.

Despite our substantially longer measurement we still have low SNR when measuring thermally polarized samples. In addition, comparing a hyperpolarized pyruvate sample to a thermal reference of a different molecule with uncertain T_1 at 6.5 mT introduces further uncertainty. Hence, we have revised our manuscript as follows:

- Added the following discussion of the uncertainty associated with our estimate to Supplementary Section 1:

Acquiring 1024 averages over 4 hours 20 minutes, our signal-to-noise is sufficient to support constrained fitting of the anticipated quartet, even though the outer components are not clearly resolved. We fit the resulting data with a quartet of Lorentzian lines, holding the relative amplitudes to the expected 1:3:3:1 ratio and only using a single amplitude variable for fitting along with linewidth, J-coupling and chemical shift for a total of 4 fit parameters. The resulting fit gives a J-coupling of 6.76 ± 0.07 Hz, agreeing well with the expected splitting despite the poorly resolved outer lines of the quartet. Using a more concentrated ^{13}C -labeled compound, or a thermal reference acquired with a significantly higher number of averages, would provide a much more robust and reliable calibration of the thermal polarization. ... The ^{13}C polarization value for our estimate calculated by this procedure is 3.45%, but to appropriately reflect the substantial uncertainty associated with our estimate we only quote it to 1 significant figure.

- Revised the MATLAB calculation script provided to include the new result, leaving the original value for the integral following 256 averages as a comment for transparency.
- Figure 1D revised with updated thermal acetic acid data with 1024 averages.
- Figure 1D legend changed " $P_{13\text{C}} = 3.3\%$ " to " $P_{13\text{C}} \sim 3\%$ " to match the estimate quoted in the abstract, and to clarify within Figure 1 that the polarization value is an estimate.
- Supplementary Figure 1A revised with updated thermal acetic acid data with 1024 averages.
- Supplementary Figure 1A caption updated to reflect the revised data and fitting procedure:

[1- ^{13}C] acetic acid spectrum acquired with 1024 averages of a 45° pulse every 15 s applied to a 31.4 mmol sample. A fit with the sum of four Lorentzians $y = (a/3)/((x - x_0)^2 + b) + a/((x - x_0 + J)^2 + b) + a/((x - x_0 + 2J)^2 + b) + (a/3)/((x - x_0 + 3J)^2 + b)$ is plotted as a solid purple line and its integral plotted in black.

R1.2

T_1 and T_2 measurements are performed under continuous pH_2 bubbling (Supplementary Figures 4 and 6). The ongoing bubbling during the acquisitions may introduce convection, susceptibility fluctuations, and residual SABRE-related polarization effects, potentially influencing the apparent mono-exponential decay, as is clearly visible in Supplementary Figure 6. The manuscript should explicitly discuss how these factors may bias the extracted relaxation times and clarify that the reported T_x values represent effective relaxation under continuous bubbling rather than intrinsic relaxation constants. Supplementary Fig. 6 suggests non-mono-exponential behavior in T_2 decay, warranting discussion of potential multi-component relaxation.

Authors' response: We appreciate the reviewer's perceptive examination of our relaxation measurements and the excellent suggestion to further discuss how ongoing bubbling during acquisitions may affect our T_1 and T_2 measurements. We have made additions to the original short discussion of these effects in the supplementary and have explicitly addressed in the main manuscript how factors

introduced by ongoing bubbling may bias the relaxation times reported. The itemized revisions are as follows:

- We have added the following text to Supplementary Section 3, from line 160 onward, in our paragraph discussing the effective T_1 :

The ongoing bubbling may introduce convection, susceptibility fluctuations, and residual SABRE-related polarization effects, potentially influencing the measured relaxation time.

- We have added the following discussion of non-mono-exponential decay observed in our Hahn-echo-style experiments at the end of Supplementary Section 3:

As with our experiments measuring T_1 relaxation, the constant bubbling occurring throughout our Hahn-echo experiments likely influences the measured relaxation time, which should similarly be considered an "effective T_2 " rather than an intrinsic relaxation constant for our SABRE solutions in the millitesla regime. The apparent non-mono-exponential behavior observed in our Hahn-echo-style experiments is probably caused by the magnetic susceptibility fluctuations from ongoing bubbling. While a spin-echo experiment can refocus field inhomogeneities over timescales slower than the echo time, at the 30 sccm pH_2 flow rate used for this measurement, bubbling creates local fluctuations in magnetic field on much faster timescales than the relevant echo time regime. Spin dephasing from these fluctuations is not refocused by the echo experiment, resulting in a steeper decay at these timescales than described by the mono-exponential curve usually observed in liquid-state NMR. Our data are better described by a Gaussian function, usually associated with decay curves from Hahn-echo experiments where inhomogeneous broadening dominates spin-spin relaxation.

- We have revised Supplementary Figure 6B with the inclusion of a Gaussian decay fit to the data, and edited the caption accordingly.
- In the discussion section of the main text we have clarified that the reported relaxation times are "effective" decay constants:

As our values were measured under continuous pH_2 bubbling conditions, convection, susceptibility fluctuations and SABRE-related hyperpolarization effects may bias the extracted relaxation times. As such, our reported T_1 represents the effective relaxation with continuous bubbling rather than the intrinsic decay constant in the millitesla regime. Further, we expect purified pyruvate solutions to exhibit longer T_1 times, particularly if D_2O is utilized as the solvent (1) and rigorous care is taken to optimize the solution composition and exclude dissolved O_2 : Recent work has shown that pyruvate T_1 times approaching 4 minutes are achievable at millitesla fields. (2)

R1.3

The MRI data are presented exclusively in arbitrary/normalized units, without any absolute quantification of noise levels or SNR. In Figures 2 and 3, the intensity scales are normalized to the noise floor, preventing the reader from assessing the true performance of the imaging system and the practical detectability of hyperpolarized ^{13}C at 6.5 mT. The manuscript should report quantitative SNR values (e.g. peak signal/standard deviation of noise in a signal-free region) or CNR values and, ideally, sensitivity expressed per μmol of ^{13}C . These metrics are essential for meaningful comparison to existing hyperpolarization and low-field MRI technologies and for evaluating how close the current implementation is to in vivo–relevant performance.

Authors' response: We welcome the opportunity to clarify the sensitivity of our ^{13}C MRI. We agree with the reviewer that quantitative SNR values are essential, and our MRI data are indeed normalized by signal/standard deviation of noise in a signal-free region. This is described at the end of the methods section but we acknowledge it is important to have this information available clearly and immediately to hand where the MRI data is presented. To clarify this normalization process, and explicitly quantify our peak SNR and the SNR within the NMR tube region, we have revised our ^{13}C MRI figures (Figures 2 and 3) and their corresponding captions. Additionally, we include a short discussion of the ^{13}C sensitivity this SNR implies and future improvements to boost sensitivity to progress towards in vivo experiments. Revisions are as follows:

- We have revised Figure 2 to include an explicit quantification of the peak signal-to-noise ratio in the central slice of the ^{13}C MRI image shown.
- Figure 3 revised as above to include peak SNR explicitly.
- Figure 2 caption revised to clarify normalization and quantify average SNR within the NMR tube region of the central slice:

All MRI data was zero-filled by a factor of 2 in the readout and first phase encode directions and normalized in units of the noise floor by dividing the signal in each voxel by the standard deviation of the noise in a signal-free region. The peak signal-to-noise ratio (SNR_{peak}) of the ^{13}C MRI is 293, while the mean SNR inside the region of the NMR tube in the central slice is 204.

- Figure 3 caption revised to clarify normalization and quantify average SNR within the NMR tube region of the central slice:

All MRI data was zero-filled by a factor of 2 in the readout and first phase encode directions and normalized in units of the noise floor by dividing the signal in each voxel by the standard deviation of the noise in a signal-free region. The peak signal-to-noise ratio (SNR_{peak}) is 613, while the mean SNR inside the region of the NMR tube in the central slice is 270.

- MRI sensitivity per μmol of ^{13}C included in the discussion section to enable quantitative comparison of our work with existing technology and allow a reader to better evaluate our results:

To quantify the current sensitivity of our system, we consider the average SNR of ~ 200 achieved in our SLIC-shot ^{13}C MRI experiment performed with $\sim 12\ \mu\text{mol}$ ^{13}C . Assuming SNR scales linearly with ^{13}C concentration, at our current ^{13}C polarization our results imply we could visualize ^{13}C above our system noise floor at concentrations down to $0.12\ \mu\text{mol}$. We note that need for discrimination of metabolic products and faster readout to minimize T_1 relaxation and maximize temporal resolution will require tradeoffs in absolute sensitivity to ^{13}C .

R1.4

The discussion section does not fully address challenges such as catalyst removal, purification, achievable bolus volumes, transfer times, and the impact of shorter T_1 at low field. A more balanced assessment of the remaining hurdles for translation would provide important context for the reader.

Authors' response: We acknowledge the importance of addressing these hurdles for translation of SABRE hyperpolarization methods and thank the reviewer for the chance to further discuss them to strengthen our manuscript. We have made the following changes to the discussion to provide a more extensive picture of the obstacles to translation and the progress to date in overcoming them:

- We have added more detail to the discussion section addressing the challenges of catalyst removal, purification, automated transfer and the gap between current preclinical prototypes and clinical dose volumes:

Using an external polarizer will address the need for larger boluses of polarized ^{13}C metabolites and incorporate automated depressurization and purification processes for rapid transfer. Recent advances have made progress on each of these steps. PHIP-SAH preclinical polarizers that can reliably deliver 2 mL doses of $\sim 20\%$ polarization at 70-180 mM concentration are available commercially. (3) SABRE-based prototypes have reached similar performance levels, crucially overcoming hurdles associated with removal of the iridium-based SABRE catalyst (4, 1) and moving away from methanol solvent towards more readily extracted acetone mixtures (5). A key future challenge is scaling up parahydrogen-based technology towards human clinical applications where larger doses at higher polarization and concentration are routinely delivered alongside more stringent quality control requirements. For example, most sites performing clinical trials with hyperpolarized pyruvate deliver doses of 220-280 mM pyruvate in volumes $>40\ \text{mL}$ and $>15\%$ ^{13}C polarization. (6) Despite clinical translation gaps, parahydrogen-based techniques show remarkable promise and have succeeded in preclinical demonstrations. Future process engineering and device automation should enable scale up of the technology.

- We have extended the discussion of T_1 relaxation. The relevant section of the corresponding paragraph in the discussion has been revised to read:

Further, we expect purified pyruvate solutions to exhibit longer T_1 times, particularly if D_2O is utilized as the solvent (1) and rigorous care is taken to optimize

the solution composition and exclude dissolved O₂: Recent work has shown that pyruvate T_1 times approaching 4 minutes are achievable at millitesla fields. (2) Future preclinical work will not only need to address sample composition but incorporate automation of purification, transfer and injection steps, to minimize T_1 relaxation between polarization and imaging.

Reviewer 2

Reviewer #2 (Remarks to the Author):

The manuscript by Boele et al, presents technically impressive work demonstrating hyperpolarized ¹³C MRI at ultra-low field and resolving chemical shift features of pyruvate and its metabolites. The imaging and spectroscopy results, particularly the ability to perform balanced steady-state readouts and distinguish spectral features at 6.5 mT, represent a meaningful contribution. The study is clearly relevant to ongoing efforts aimed at reducing costs and increasing accessibility in metabolic MRI. However, several conceptual points require clarification, and the narrative would benefit from realignment. In its current form, the manuscript emphasizes aspects that are not central to the actual novelty, while providing insufficient clarity on the broader translational context and on what this work does (and does not) address.

Authors' response: We are grateful to the reviewer for highlighting the technical accomplishments of our work and its contribution to the field. We welcome their constructive feedback that we have incorporated into our manuscript, improving the clarity of how our work is positioned within a broader translational context. Our point-by-point response follows.

Major comments:

1. In the abstract and introduction, the authors state that the limited use of hyperpolarized ¹³C MRI in the clinic is “primarily due to a lack of access to affordable technology.” This is not accurate and misrepresents the current state of clinical translation. Dynamic nuclear polarization equipment is indeed expensive, but cost has not been the principal barrier to adoption. It's the lack of evidence demonstrating that hyperpolarized ¹³C measurements improve diagnosis or guide treatment decisions that is missing. The field is still establishing clear, validated clinical indications. Cost becomes a secondary factor once a clinical indication is established, as the authors themselves have previously demonstrated with ultra-low-field proton MRI. Please reframe the rationale to reflect that the main challenge is the absence of fully established clinical use-cases; cost reduction is valuable but not the determining obstacle.

Authors' response: We thank the reviewer for the opportunity to clarify the translational context of hyperpolarized ¹³C metabolic MRI. We agree that the primary barrier to clinical translation is the establishment of clear, validated clinical benefit, and that cost considerations become decisive only once such benefit has been demonstrated. We acknowledge that our original manuscript did not sufficiently distinguish between these factors and therefore overstated the role of cost in limiting clinical adoption. We have revised the manuscript to explicitly position cost and accessibility as

secondary considerations that constrain the scale and throughput of the research needed to establish clinical indications, and to provide clearer translational context. The specific changes are outlined below:

- We have revised the abstract to reflect the reviewer's point that the primary barrier to widespread clinical adoption remains the lack of established, validated clinical indications. We now present cost and accessibility as secondary factors that limit the pace and breadth of the research required to establish those indications. The revised sentence now reads:

Hyperpolarized ^{13}C -enriched pyruvate has demonstrated promise for imaging metabolism in cancer, heart disease and neurodegenerative disorders; however, broader clinical adoption awaits validated clinical indications, and is further constrained by the cost and limited availability of current hyperpolarization technology.

- We have revised the corresponding paragraphs in the Introduction to realign the narrative around clinical validation as the decisive step, while retaining the discussion of how current DNP-based infrastructure constrains the scale and throughput of studies needed to establish clinical utility. The revised text now reads:

However, clinical research with hyperpolarized ^{13}C MRI has been largely confined to a small number of centers worldwide. To date, there remains insufficient evidence that hyperpolarized ^{13}C MRI improves diagnosis or guides treatment decisions across established clinical pathways. Establishing validated clinical indications is therefore the primary requirement for widespread adoption. In parallel, the cost and limited availability of current hyperpolarization platforms—predominantly based on dissolution dynamic nuclear polarization (DNP)—constrain the scale and throughput of the clinical and translational studies needed to generate this evidence. (7) ... These practical limitations lead to slow throughput and high costs for DNP-based workflows (e.g., ~1 h per sample for commercial systems costing > \$2M), restricting the breadth of research required to validate clinical utility and define robust use cases for ^{13}C MRI.

2. Much of the manuscript, focuses on the specifics of SLIC-SABRE polarization. While SLIC-SABRE is the chosen method for creating the hyperpolarized ^{13}C pyruvate enhancement, the main technical novelty of the present work is ultra-low-field imaging and spectral discrimination, not the polarization mechanism. Clarify explicitly why SLIC-SABRE is discussed at this level of detail, if there is a specific scientific justification, it should be stated; if not, significant shorten the detailed information of SLIC-SABRE. A short paragraph acknowledging the method's cost-effectiveness and compatibility with low fields should suffice.

Authors' response: We thank the reviewer for their welcome broader perspective and concede that we have perhaps focused too much on the specifics of SLIC SABRE polarization and the positioning of the technical advances of our work within the context of SABRE research. To better focus our discussion on the main novelty of our work while still acknowledging the work of others in the field we have moved the paragraphs on SLIC SABRE to a new Supplementary section, and revised the

discussion to emphasize the potential of ^{13}C MRI at ULF over the opportunities specific to SABRE methods at 6.5 mT. The specific revisions are as follows:

- We have moved the paragraphs extensively comparing SLIC SABRE at mT fields to prior work in the T and μT regimes to a new section in the Supplementary (Section 6). To maintain proper acknowledgment of previous relevant research in the main text we have moved any affected citations to alternative appropriate locations.
- We have added the following text to the discussion and edited the relevant subheading to "SABRE in the mT regime" to reflect the reframing of this subsection of the discussion:

SLIC SABRE is our chosen hyperpolarization method primarily because it generates reproducible ^{13}C enhancement in situ. (8, 9, 10) Further advantages of performing SLIC SABRE at mT magnetic fields, compared to the T and μT regimes, are discussed in more detail in Supplementary Section 6. More generally, the cost effectiveness of SABRE hyperpolarization and its compatibility with 6.5 mT are our key motivations for pursuing applications of SABRE methods.

Minor:

1. Add a short paragraph acknowledging that a clinical indication for ^{13}C pyruvate MRI at low field remains to be established and while cost-efficient hyperpolarization and ultra-low-field systems are valuable, validation of the clinical utility at high field is the decisive next step. You should cite the consensus for hyperpolarized ^{13}C for performing the necessary trials, that will pave the way for clinical utilization.

Authors' response: We thank the reviewer for this excellent suggestion. We have revised our discussion accordingly to better explain the translational pathway for hyperpolarized ^{13}C MRI. We have added the following short paragraph:

Considering the clinical translation of hyperpolarized ^{13}C MRI at low field, we acknowledge that a clinical indication remains to be established. However, cost-effective parahydrogen-based hyperpolarization methods will accelerate research to establish clinically validated use cases for ^{13}C MRI. The decisive next step will be clinical validation at high field through further clinical trials. (11, 6) Following high-field validation, a compatible low-field use case must leverage the advantages of portable, low-cost MRI to expand access to ^{13}C MRI.

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