Magnetic Resonance Thermometry of Flowing Blood

Chinthaka C. Heyn, Jonathan Bishop, Kyle Duffin, Wayne Lee, Jun Dazai, Shoshana Spring, Brian J. Nieman, and John G. Sled

Version Post-print/accepted manuscript


Publisher's Statement This is the peer reviewed version of the following article:
which has been published in final form at [Link to final article using the DOI]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

How to cite TSpace items

Always cite the published version, so the author(s) will receive recognition through services that track citation counts, e.g. Scopus. If you need to cite the page number of the author manuscript from TSpace because you cannot access the published version, then cite the TSpace version in addition to the published version using the permanent URI (handle) found on the record page.

This article was made openly accessible by U of T Faculty. Please tell us how this access benefits you. Your story matters.
MR Thermometry of Flowing Blood

Chinthaka C. Heyn1,2,3†, Jonathan Bishop1†, Kyle Duffin4, Wayne Lee5, Jun Dazai1, Shoshana Spring1, Brian J. Nieman1,6,7, John G. Sled1,6

1Mouse Imaging Centre (MICe), Hospital for Sick Children, Toronto ON, Canada
2Department of Medical Imaging, University of Toronto, Toronto ON, Canada
3Physical Sciences, Sunnybrook Research Institute, Toronto, ON, Canada
4Centre for Phenogenomics, Toronto ON, Canada
5Neuroscience and Mental Health, Hospital for Sick Children, Toronto, ON, Canada
6Department of Medical Biophysics, University of Toronto, Toronto ON, Canada
7Ontario Institute for Cancer Research, Toronto ON, Canada
†Authors contributed equally to this work

Running Head: MR Thermometry of Flowing Blood

Please address correspondence to:
John G. Sled
Mouse Imaging Centre
Hospital for Sick Children
555 University Avenue
Toronto, ON Canada M5G 1X8
Phone: 416 813-7654 x309557
Fax: 647 837-5832
Email: john.sled@utoronto.ca

Word Count: 4,526
ABSTRACT

Blood temperature is a key determinant of tissue temperature and can be altered under normal physiological states such as exercise, in diseases such as stroke, or iatrogenically in therapies which modulate tissue temperature such as therapeutic hypothermia. Currently, available methods for measuring arterial and venous temperatures are invasive, and for small animal models are impractical. Here, we present a methodology for measuring intravascular and tissue temperature by MRI using the lanthanide agent TmDOTMA-. The approach makes use of phase sensitive imaging measurements combined with spectrally selective excitation to monitor the temperature dependent shift in the resonance of proton nuclei associated with water and with methyl groups of TmDOTMA-. Measurements were first made in a flow phantom modelling diastolic blood flow in the mouse aorta or inferior vena cava (IVC) and imaged using a 7 T pre-clinical MRI with a custom-built surface coil. Flowing and static fluid temperatures agreed to within 0.12°C for these experiments. Proof-of-concept experiments were also performed on three healthy adult mice demonstrating temperature measurements in the aorta, IVC and kidney following a bolus injection of contrast agent. A small (0.7-1°C) but statistically significant higher kidney temperature compared to aorta (p=0.002 to 0.007) and IVC (p=0.003 to 0.03) was shown in all animals. These findings demonstrate the feasibility of the technique for in vivo applications and illustrate how the technique could be used to explore the relationship between blood and tissue temperature for a wide range of applications.

Abbreviations: tetramethyl-1,4,7,10- tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTMA); inferior vena cava (IVC); specific absorption rate (SAR)

Keywords: Magnetic resonance imaging, Thermometry, Flow, TmDOTMA
MR thermometry is an indispensable tool that non-invasively measures the temperature of tissue for a growing number of applications. MR thermometry based on temperature dependent changes of the proton resonance frequency (PRF) is used clinically for monitoring heating or cooling during ablation therapies with high-intensity focused ultrasound\(^1\), radiofrequency\(^2\), laser\(^3\) and cryotherapy\(^4\). The technique is also increasingly used as a research tool for estimating specific absorption rate (SAR)\(^5,6\) for radiofrequency pulses used in MRI as well as tissue temperature mapping in a range of disease conditions including stroke\(^7,8\), cancer\(^9\), and neurodegenerative disease\(^10\).

*In vivo* tissue temperature depends on a number of factors including endogenous heat production from tissue metabolism, exogenous heat deposition such as from radiofrequency power deposition (e.g. SAR), and the transfer of heat to or from the tissue by heat conduction to the environment or convection via blood flow. While there are a number of bioheat exchange models to describe the relationship between these factors, the Pennes bioheat exchange equation is perhaps the most well-known\(^11\) and has been adapted to account for SAR\(^6\):

\[
\rho c \frac{\partial T}{\partial t} = Q + \rho SAR + \nabla \cdot (k \nabla T) - W \rho_{bt} c_{bt} (T - T_{bt}) \quad \text{[Eq. 1]}
\]

\(T\) is tissue temperature, \(\rho\) is tissue density, \(c\) is specific heat capacity, \(Q\) is heat generated by tissue metabolism, SAR is the specific energy absorption rate, \(k\) is thermal conductivity, \(W\) is tissue blood perfusion and \(bt\) subscript denotes quantities related to arterial blood. Historically, arterial blood temperature was measured directly by invasive arterial measurement\(^11\). A non-invasive MR
thermometry methodology for measuring blood temperature would provide a more convenient
evaluation of bioheat exchange and could also provide further insight into bioheat exchange under
conditions where blood temperature is changing such as during therapeutic hypothermia\textsuperscript{12} or
exercise\textsuperscript{13} for instance.

Recently there has been growing application of MR thermometry using lanthanide chelates
such as Thulium (III) (1R,4R,7R,10R)-\(\alpha,\alpha',\alpha'',\alpha''''\)-tetramethyl-1,4,7,10- tetraazacyclododecane-
1,4,7,10-tetraacetic acid (TmDOTMA-) for measuring tissue temperature \textit{in vivo} with high
precision\textsuperscript{14-16}. Compared to thermometry based on the PRF shift of water, the resonance frequency
of the TmDOTMA- ligand is particularly sensitive to temperature, approximately 60 times greater
than the PRF of water for the methyl resonance. In addition, unlike other temperature sensitive
lanthanide chelates such as Thulium (III)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis-
(methylene phosphonate) (TmDOTP\textsuperscript{5}), TmDOTMA- is an ideal agent for thermometry as it is
insensitive to changes in tissue pH and tissue ion concentration and has 12 equivalent methyl
protons to provide high SNR\textsuperscript{14}. In the present work, we build upon previous applications of MR
thermometry with TmDOTMA- and demonstrate phase-based temperature measurements in
flowing blood. We first validate this technique in a phantom modeling diastolic blood flow in the
mouse aorta or inferior vena cava (IVC) and then apply the methodology to non-invasively
quantify the temperature of flowing blood \textit{in vivo} in the mouse abdomen using a standard dose
(~0.7 mmol/kg) of TmDOTMA-.

\section*{METHODS}

\subsection*{Phantom Design}

\clearpage
A flow phantom was constructed using a 1 mm inner-diameter glass capillary tube connected on each end to 50 mL reservoirs using plastic tubing (Figure 1a). The system was filled with 20 mL of TmDOTMA- (Macrocyclics, Dallas, TX, USA) dissolved in 1X PBS to a concentration of 25 mM and gas bubbles were carefully flushed out of the system. Two fibre optic temperature sensors (OTG-M3000, Opsens, Quebec City, QB, Canada) were positioned within the tubing just proximal and distal to the glass capillary tube. These probes are reported to have a 0.01°C temperature precision (standard error of mean, SEM) and a 0.7°C accuracy. The temperature readings between the probes in our setup were within 0.2-0.3°C of each other in static and flowing conditions. The temperature of fluid within the glass capillary tube was taken to be the mean of these two temperature readings. Flow through the system was produced by injecting air into one of the reservoirs using an injector pump positioned outside the MR suite (Harvard Apparatus, Holliston, MA, USA). The system was run in reverse to refill the reservoir prior to each run. The temperature of the magnet bore was maintained by blowing warmed air within it using a commercial pet grooming stand dryer (Edemo Dryers Inc., Peyton, CO, USA) controlled using a t-type thermocouple and temperature controller (Omron Corp., Kyoto, Japan). Prior to each flow experiment at a specific temperature, the system was allowed to thermally equilibrate until the temperature reading in each temperature probe was constant in static and flowing conditions (typically approximately 30 minutes for each 1°C temperature change). The glass capillary tube was positioned on a 5×3.5 cm (length×width) custom-built surface coil with 5 mm spacer between the coil and tube.

The phantom design was selected to allow both water proton and TmDOTMA- temperature measurements with non-selective spectroscopic and phase-based imaging methods. As such, we intentionally chose not to immerse the capillary tube in fluid or gel. The non-selective
spectroscopic temperature measurements (in both water and TmDOTMA-) were taken as the gold-standard and provided a reference for the phase-based experiments. Addition of fluid or gel outside the capillary would prevent non-selective spectroscopic water proton measurements because signal would arise from both inside (flowing) and outside (static) of the tube. The presence of fluid or gel outside the capillary tube, however, does not affect results of the phase-based experiment, which can also be applied in vivo.

Animal Preparation

In vivo experiments were conducted according to an institutional animal use protocol with normal CD1 mice of approximately 40 g weight (n=3). This strain and weight were chosen for the larger tail vein and comparative ease of catheterization. The mouse was warmed under a heat lamp for 30 minutes before the procedure. Anesthesia was achieved with intraperitoneal (IP) administration of 100 mg/kg ketamine and 10 mg/kg xylazine. The mouse was placed on a heating pad and immobilized with tape and eye salve applied to prevent drying. Under aseptic conditions, a 3/4” 26 G IV catheter (Abbocath, Hospira, Saint Laurent, QB, Canada) was primed with a saline/heparin solution and inserted into the tail vein. A syringe containing TmDOTMA- solution at a concentration of 0.2 M (prepared in 1X PBS and filtered) was then attached. Two capillary tubes of 10 mM TmDOTMA- were taped onto the ventral aspect of the animal for imaging reference purposes. In the bore of the magnet, the mouse was kept warm with a flow of hot air at a temperature of 29°C. The animal was positioned prone on a loading bed and entered headfirst in the RF coil. Respiration was monitored with a pneumatic pillow (SAII, Stony Brook, NY, USA).

Temperature Calculations
The chemical shift difference between the temperature sensitive methyl resonance of TmDOTMA- and the proton resonance of water can be approximated by the following linear expression:

\[
\delta_{\text{TmDOTMA}} - \delta_{\text{H2O}} = C_T \cdot T + C_0 \quad [\text{Eq. 2}]
\]

where \( C_T \) is a constant measured in ppm/°C, \( T \) is temperature in °C, and \( C_0 \) is a constant that represents the predicted chemical shift difference between TmDOTMA- and water at 0°C. \( \delta_{\text{H2O}} \) and \( \delta_{\text{TmDOTMA}} \) are the proton resonances associated with water and TmDOTMA- respectively. Equation 2 ignores the small temperature dependence of \( \delta_{\text{H2O}} \) which will result in an underestimation of \( C_T \) by 0.01 ppm/°C. Equation 2 also ignores the small higher order temperature dependence of \( \delta_{\text{TmDOTMA}} \) resulting from dipolar paramagnetic interactions\(^{17}\). The linear relationship described by Equation 2 has been confirmed experimentally over a wide temperature range in phantoms and \textit{in vivo} and the \( C_T \) constant for the methyl resonance of the major isomer of TmDOTMA- has been measured by a number of groups with reported values ranging from 0.57\(^{14}\) to 0.7 ppm/°C\(^{18}\). For the remainder of this paper and for simplicity of discussion, all references to TmDOTMA- will refer to the methyl resonance of the major isomer and not the other minor nuclear resonances of the DOTMA\(^+\) ligand\(^{14}\).

By acquiring two phase images, one for water and one for the methyl TmDOTMA- resonance using spectrally selective excitation in conjunction with limited receiver bandwidth that excludes other proton resonances on the DOTMA\(^+\) ligand (eg. \( H_1 \) to \( H_5 \) protons), the temperature of static or flowing TmDOTMA- can be computed on a voxel-by-voxel basis from residual temperature phase shifts using the following formula\(^{15}\):
\[
\delta_{\text{TmDOTMA}} - \delta_{\text{H2O}} = \frac{1}{f_0} \left[ t_{\text{of TmDOTMA}} - t_{\text{of H2O}} + \frac{\Delta \phi_{\text{TmDOTMA}}}{2\pi \tau_{\text{TmDOTMA}}} - \frac{\Delta \phi_{\text{H2O}}}{2\pi \tau_{\text{H2O}} \cdot \phi_{\text{GHIJGKL}}} \right] \quad \text{[Eq. 3]}
\]

where \(f_0\) is the nominal proton reference frequency for the scanner (299.4 MHz at 7 T), \(t_{\text{of TmDOTMA}}\) and \(t_{\text{of H2O}}\) are the receive offset frequencies for the TmDOTMA- and water acquisitions respectively, \(\Delta \phi_{\text{TmDOTMA}}\) and \(\Delta \phi_{\text{H2O}}\) is the phase accrual for TmDOTMA- and water (in radians) during the respective time periods \(\tau_{\text{TmDOTMA}}\) and \(\tau_{\text{H2O}}\).

MRI

Phantom Experiments

MRI was performed on a 40 cm-diameter bore, 7 T magnet (Magnex Scientific, Oxford, UK) and DirectDrive console (Agilent Technologies, Santa Clara, CA, USA). The capillary tube within the phantom was shimmed to approximately 20 Hz linewidth, and reference frequencies for water and TmDOTMA- were obtained. In order to ascertain the calibration coefficients \(C_T\) and \(C_0\) (Equation 2) for static and flowing TmDOTMA-, a spectroscopic experiment was performed over a range of temperatures for static and flowing TmDOTMA-. Parameters for this acquisition on TmDOTMA- were number of transients, \(n_t=512\), receiver bandwidth=10000 Hz, number of points, \(n_p=400\), \(T_R=30 \text{ ms}\), flip angle=90°, slice thickness=10 mm, while on the water resonance averages were reduced to 4. We did not employ selective water suppression due to the substantial chemical shift of the TmDOTMA- resonance from water. With the 800 \(\mu\text{s}\) shaped pulse width of the slab selective excitation, the RF bandwidth centered at the TmDOTMA- excitation frequency did not have significant amplitude at the water resonance, and in addition with the slice selection gradient amplitude of 0.8 G/cm, the spatial location of any residual water excitation at the TmDOTMA-
excitation frequency was calculated to be 8.8 cm and beyond the physical extent of the capillary phantom.

Temperature of static and flowing fluid was obtained using a phase evolution experiment utilizing calibration coefficients derived spectroscopically. The experiment was conducted as single-slice acquisition with 20×20 matrix and 10 mm slab thickness for a resolution of 1×1×10 mm. The capillary tube was visualized in the through-slab direction in a single voxel of the field-of-view. In the case of TmDOTMA- imaging, nt=56, TR=20 ms, flip angle=90° and four echo times 1.4, 2.4, 3.4 and 4.4 ms were obtained over a scan duration of 1 minute 30 seconds. Water images were also obtained at the same resolution using nt=4, TR=20 ms, flip angle=15° with echo times 3, 6, 9, and 12 ms for a total of 6 seconds. The TmDOTMA- reference frequency was determined from an FID experiment on a 1 cm slab after each temperature change. The transmit frequency for TmDOTMA- was kept on resonance but the receiver offset frequency was set to +200 Hz off resonance before each experiment to prevent aliasing and the ambiguity of a null phase measurement. The water reference frequency was left unchanged. Given the target velocities used in this experiment and the comparatively small first moment of the slab-select gradient with the 10 mm slab, the expected phase accrual due to flow through the slice select gradient was calculated to be 0.017 rad or 6.4 x 10⁻³ ppm shift in water resonance frequency for 1.4 ms echo time. Given this small effect, flow compensation was not performed, thus minimizing echo time and maximizing SNR. A total of three flow passes were obtained for each temperature set-point.

To test temperature measurements under different flow rates, the phase experiment described above was repeated at a constant temperature as determined by fibre optic temperature measurements. Flow velocity in the capillary tube was measured in water with an unbalanced
phase contrast acquisition in a transverse slice with the capillary tube visualized in cross-section, and flow encoding employed along the axial direction of the tube. The velocity encoding was set to detect maximum flow of 15 cm/s, and an imaging matrix of 100×100 was used for voxel resolution of 0.2×0.2×1 mm. Eddy current correction was performed by subtracting the small net phase measured for the static condition from the phase calculated for each flow measurement. Maximum flow velocities in the range of 2.5 to 3.0 cm/s were attainable and comparable to diastolic blood flow in major arteries and veins in mice\textsuperscript{19}.

\textit{In-vivo Experiments}

After first-order linear shimming, localizer images of the IVC in transverse orientation near the kidney were obtained, followed by pre-contrast imaging. The mouse was then removed from the bore still positioned on the bed and the TmDOTMA- injected by hand over the course of about 10 seconds to obtain an \textit{in vivo} dose of 0.7 mmol/kg. This falls within the range of previously used rodent doses of TmDOTMA- (0.5 mmol/kg\textsuperscript{18} – 1.4 mmol/kg\textsuperscript{15}). The mouse was then immediately returned to the bore to the same position and several post-contrast image sets were obtained.

MR imaging was conducted using a 30 mm quadrature volume coil (Varian Inc., Palo Alto, CA, USA) using a GRE sequence with TR=3.5 ms and a minimum TE=1.3 ms. Each image set pre- and post-contrast consisted of transverse water proton and three separate TmDOTMA- images with echo times of 1.3, 1.55 and 1.8 ms. The voxel resolution was 1.6×1.6×10 mm in a 3.2 cm FOV. For water proton images nt=2 acquisitions were accumulated at each echo time, while for TmDOTMA- the accumulation increased with echo time as nt=200,300,500 to maintain the SNR at an approximately constant level. A flip angle of 15° was used for water proton images and 90° for TmDOTMA-. Transmit/receive frequencies for water were assigned from the routine prescan.
For TmDOTMA-, they were assigned based on the expected frequency of TmDOTMA- methyl resonance at physiologic temperature of 37.6°C in order to maximize imaging time particularly during the period immediately after contrast administration when TmDOTMA- blood concentrations were highest.

**Image Processing and Statistics**

For the spectroscopic experiments, resonance frequencies for water and TmDOTMA- were determined using the VnmrJ 2.2C software environment. Absorption mode spectra were smoothed with line broadening (40 Hz), Fourier interpolated to 2.4 Hz resolution and automatically phase aligned before frequency assignment. For the phase evolution experiment, customized code was written in MATLAB (Natick, MA, USA) to calculate the chemical shift difference between water and TmDOTMA- according to Equation 3. For *in vivo* experiments, the high-resolution localizer and the low-resolution pre- and post-contrast image data sets were registered for incidental shifting of the mouse's position during injection by using control points in the reference capillary tubes. ROIs were drawn for aorta, IVC, kidney and the reference capillary tube. Where necessary, phase unwrapping was performed in the two spatial and one time dimension. Frequency was assigned by a least-squares fit to the phase evolution for the entire collection of pixels in each ROI, and converted to temperature using Equation 2. In addition, a pixel-by-pixel temperature map was generated after averaging all the post-contrast image sets together. Statistical analysis was performed in Prism Version 6.0 (GraphPad Software, La Jolla, CA, USA).
RESULTS

The chemical shift difference between water and TmDOTMA- measured from the spectrum of static and flowing fluid (mean flow velocity=2.5 cm/s) at several temperatures was calculated (Figure 1). The predicted linear relationship between the chemical shift difference and temperature was found for static and flowing fluid ($R^2 = 0.9996$ and 0.9980 respectively). The calculated slopes ($C_T$) for static and flowing conditions were $0.65\pm0.0038$ and $0.65\pm0.0081$ ppm/°C respectively and $C_0$ were -125.10±0.10 ppm and -124.90±0.22 ppm respectively. No significant difference in $C_T$ was found (ANCOVA, p=0.56) for static and flowing fluid. There was a small difference in $C_0$ which was significantly different (ANCOVA, p=0.0028).

Temperature imaging of flowing fluid was performed by measuring the phase evolution of TmDOTMA- (Figure 2). The flow velocity was calculated, using phase contrast methodology for water. The velocity profile within the 1 mm inner-diameter capillary tube demonstrated a typical laminar flow pattern. The in-plane resolution for the TmDOTMA- phase evolution experiment was 1×1 mm and the capillary tube therefore occupies one voxel in the center of the field of view. The offset frequencies for static and flowing TmDOTMA- calculated from the phase evolution experiment were $187 \pm 12$ Hz and $182 \pm 13$ Hz respectively (expected frequency offset 200 Hz based on the preset TmDOTMA- reference frequency).

To evaluate the performance of phase based thermometry for flowing TmDOTMA-, the temperature calculated using MR thermometry was compared to temperature measured using a fibre optic probe over a range of temperatures for mean velocity of 2.5 cm/s (Figure 3). The calibration coefficients $C_T$ and $C_0$ for flowing TmDOTMA- ascertained earlier using spectroscopy
were used. There was good agreement between the two temperature measurements ($R^2 = 0.9957$) with a slope close to unity (1.02 ± 0.019) and intercept close to zero (0.011 ± 0.48).

The effect of flow velocity on phase-based MR thermometry was evaluated at constant temperature. Flow velocities were estimated using phase contrast methodology and the mean flow velocity and maximum flow velocity for the capillary tube cross section were determined. Table 1 shows the mean chemical shift difference between water and TmDOTMA- using phase methodology and corresponding temperature estimates at different flow velocities. The mean chemical shift measured using the phase methodology was not significantly different between different flow velocities (one-way ANOVA, $p=0.065$). The mean temperature estimates by MR thermometry were slightly lower than the expected temperature (25.5°C based on fibre optic temperature measurement).

*In vivo* imaging after intravenous bolus administration of TmDOTMA- demonstrates the feasibility of detecting TmDOTMA- in the aorta, IVC and kidney for a representative animal (Figure 4). A plot of signal to noise ratio (SNR) of the methyl resonance for TmDOTMA- for this animal (Figure 5a) shows maximal signal in all compartments at the first time point (3 minutes) with subsequent clearance of contrast agent. Assuming first order elimination from the blood pool, the calculated half-life of TmDOTMA- in the aorta and IVC were 7.6 and 7.2 minutes respectively. The SNR of the reference tube containing 10 mM TmDOTMA- was ~52. Based on the relative SNR between this reference tube and *in vivo* SNR, the estimated concentration of TmDOTMA- in the aorta at 3 minutes was ~3 mM. The paramagnetic nature of TmDOTMA- results in temporally changing susceptibility related signal loss for water (Figure 5b) in each compartment. By measuring the phase evolution of TmDOTMA- and water in each tissue compartment, the temperature was measured for aorta, IVC and kidney (Figure 6). For the representative animal,
the mean temperature of the aorta, IVC, kidney and reference tube over 24 minutes was 34.1°C (σ=0.7), 34.3°C (σ=0.3), 35.0°C (σ=0.5), and 34.5°C (σ=0.1) respectively. Data for all mice is shown in Table 2. A statistically significant higher kidney temperature was found compared to aorta (Student’s t-test, p=0.002 to 0.007) and IVC (Student’s t-test, p=0.003 to 0.03) for all three animals.

**DISCUSSION**

In the present study, we demonstrate the feasibility of non-invasive temperature measurement of flowing blood, an application of MR thermometry which has not been described previously. The methodology uses the contrast agent TmDOTMA- which is not approved for human use so that the technique described herein is presently limited to animal experiments only. A spectroscopic methodology was first used to evaluate the relationship between TmDOTMA- chemical shift and temperature for static and flowing contrast agent in a phantom. This methodology was used to confirm the linear chemical shift-temperature relationship for TmDOTMA- for static and flowing fluid and to calculate calibration values $C_T$ and $C_0$ which were used for subsequent phase-based temperature calculations.

The calculated calibration coefficients for static and flowing TmDOTMA- were similar to those measured by others $C_T$ = 0.64 ppm/°C and $C_0$ = -124.7 ppm$^{15}$. The $C_T$ and $C_0$ for TmDOTMA-calculated for static and flowing fluid were in good agreement aside from a small statistically significant difference for $C_0$. This small difference in $C_0$ could result in an underestimation of temperature in static tissues (by ~0.3°C) when $C_0$ for flowing conditions are utilized.
MR thermometry of flowing TmDOTMA- was performed using a phase evolution experiment that has been previously described for measuring temperature of static TmDOTMA- in phantoms and tissues\textsuperscript{15}. Using this technique, good agreement was found between MR thermometry of flowing TmDOTMA- and temperature measurements made with a fibre optic sensor. The methodology was also tested over different flow rates and showed reproducible temperature measurements over the small range of flow velocities examined. We did observe a 0.5°C lower temperature compared to fibre optic temperature measurements which was within the error (accuracy) of the fibre optic thermometer.

There were several limitations of the phantom experiments. First, we conducted our phantom calibration experiments at less than physiologic temperatures. To achieve higher temperatures in the phantom, design changes to minimize heat loss and enhance temperature uniformity at higher temperatures would be required. However, measurements made in the 24-30°C temperature range are expected to be applicable at physiologic temperatures, provided the temperature-frequency relationship is linear over this range. Support for this comes from experimental data which has shown negligible contribution of higher-order temperature terms to C\textsubscript{T}\textsuperscript{17} and a strong linear relationship (R\textsuperscript{2}=0.998) between TmDOTMA- chemical shift and temperature over a larger temperature range (25-55°C) than what was considered in our phantom experiments\textsuperscript{14}. Second, the flow rates that were explored in the phantom study were comparable to flow rates that are observed during diastole in major arteries and venous structures\textsuperscript{19} of the mouse, but did not extend to higher rates observed during systole. We considered these phantom measurements to be physiologically relevant particularly given that diastole occupies the majority of the cardiac cycle. Again, design changes would be necessary for the phantom to increase driving pressure (or reduce flow resistance) to obtain higher flow rates in the phantom. Finally, the
concentration of TmDOTMA- used in the phantom experiments was on the high end of expected blood concentration. The concentration used was based on biodistribution of a dose of 1.5 mmol/kg within total blood volume assuming a total mouse blood volume of 58.5 ml/kg which is the maximal theoretical blood concentration attainable. This dose was chosen based on previous experiments in rats. Previous pilot in vivo experiments that we conducted at this high dose in mice, however, resulted in high incidence of death shortly after bolus administration and provided motivation for reducing the dose used in subsequent in vivo experiments.

The phantom experiments aided the design of the in vivo experiments. Given the lower concentration of TmDOTMA- that could be achieved in vivo, we were limited in the voxel resolution that could be achieved. Slice thickness remained at 10 mm which only allowed temperature quantification in large vessels. Furthermore, we reduced the spatial resolution from 1 mm\(^2\) in plane used in the phantom experiments to 1.6 mm\(^2\) and reduced the echo spacing from 1 ms to 0.25 ms. With these modifications and use of quadrature volume coil, we were able to achieve sufficient SNR to quantify blood temperature in the IVC and aorta over a 24 minute period. We did not explicitly study the relationship between decreasing TmDOTMA- SNR and temperature uncertainty which is expected to worsen as SNR decreases\(^2\). Also, our data shows an inverse relationship between water signal and TmDOTMA- concentration for blood pool which is presumably the result of susceptibility related signal losses caused by TmDOTMA-. Water signal in kidney was slightly time shifted relative to blood pool and the reason for this is not entirely clear and possibly the result of renal excretion of TmDOTMA-. Further work is needed to better characterize these effects.

Our results demonstrate in vivo blood temperature consistent with mild hypothermia which is expected after anesthesia with ketamine/xylazine\(^3\). It is important to recognize that previous
authors have typically ligated the kidney to prolong \textit{in vivo} imaging time\textsuperscript{15} which was not required for the present experiments. This allowed us to evaluate the temperature of the kidney which has not been previously explored with this methodology. We observed a small but statistically significant difference in mean temperature between blood and kidney. The kidney is one of the most metabolically active organs in the body with a metabolic rate approximately twice that of brain (in humans, 440 kcal/kg/day versus 240 kcal/kg/day respectively)\textsuperscript{22}. Using invasive methods, others have reported a 0.3°C difference between arterial blood and brain\textsuperscript{23}. It was therefore not unexpected that we were able to observe a temperature differential between blood and kidney. Given that efficient dissipation of metabolic heat by tissue blood flow and/or conduction to the environment according to Equation 1 will act to reduce differences in tissue and blood temperature, in situations where there is uncoupling between metabolic rate and blood flow, greater temperature deviations between blood and tissue would be expected. For example, this has been demonstrated in brain under conditions of misery perfusion where higher brain temperatures result from impaired dissipation of metabolic heat\textsuperscript{24}. One future application of this methodology will be to explore the relationship between blood flow, blood temperature and tissue temperature in disease.

In any thermometry experiment involving metabolic temperatures, SAR heating is a concern. We did not observe a statistically significant rise in tissue temperature during the course of the experiment. To estimate the SAR for this experiment, we saw that the flip angle calibration returned a 90 degree flip angle power of approximately 90 mW during a 900 µs square pulse. At the performed TR of 3.5 ms, and allowing some power reflection due to imperfect impedance matching as well as stray E field outside the mouse, we estimate a continuous power deposition of 10 mW (SAR 0.25 W/kg for 40 g mouse). This translates into a total heat deposition of 9 J over the course of a 15-minute experiment. Assuming no losses of heat to the environment during this
time—a very conservative assumption—and an average heat capacity of the mouse equivalent to water (4.184 J °C g⁻¹), this would result in a temperature increase of approximately 0.05°C for a 40 g mouse. It is therefore not surprising that we did not observe a rise in tissue temperature due to SAR during the course of the imaging experiment.

While lanthanide chelates like TmDOTMA- are not clinically approved, the lethal dose (LD₅₀) and toxicity profile of these agents in animals are comparable to clinically approved gadolinium chelates¹⁴. Furthermore, recent advances have shown how the blood half-life of lanthanide agents can be improved by co-infusion with Probenecid which is a clinically approved inhibitor of organic anion transporters used to decrease renal clearance of drugs²⁵. This was done in rats using TmDOTP⁵ and allowed the investigators to measure brain tumor pH without the need for kidney ligation which was a limitation of the technique which reduced its potential for human use. A low toxicity profile, favorable pharmacokinetics and growing applications including the possibility of measuring tissue metabolism through evaluation of tissue bioheat exchange will contribute toward the feasibility of lanthanide contrast agents and motivate their future translation into clinical practice.

CONCLUSION

Non-invasive measurement of temperature within different blood compartments has the potential to provide important insight into tissue temperature regulation in health, disease and therapy. The measurement technique presented here has the necessary sensitivity and resolution to monitor temperature in major vessels in the mouse and can therefore leverage a wealth of information relevant to murine models. Further improvements to MR hardware (eg. customized RF coils), pulse sequence design, and contrast agent formulation which improve SNR will increase the
number of resolvable vessels and allow detection of smaller temperature differentials that exist under both physiological and pathological conditions.

ACKNOWLEDGEMENTS

Funding for this work was provided by Canadian Institutes of Health Research Grant (MOP231389 to J.G.S).
REFERENCES


### Table 1: Effect of flow velocity on chemical shift estimated temperature. $\sigma$ (standard deviation) and SEM (standard error of mean=$\sigma/\sqrt{n}$).

<table>
<thead>
<tr>
<th>Mean (Max.) flow velocity ± $\sigma$ cm/s</th>
<th>$\delta_{\text{H}<em>2\text{O}} - \delta</em>{\text{TmDOTMA}}$ ± SEM ppm</th>
<th>Temperature ± SEM °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-108.69 ± 0.052</td>
<td>(24.94 ± 0.079)</td>
</tr>
<tr>
<td>0.39 (1.84) ± 0.62</td>
<td>-108.61 ± 0.011</td>
<td>(25.06 ± 0.017)</td>
</tr>
<tr>
<td>0.87 (2.08) ± 0.64</td>
<td>-108.77 ± 0.032</td>
<td>(24.82 ± 0.050)</td>
</tr>
<tr>
<td>2.48 (5.19) ± 1.51</td>
<td>-108.65 ± 0.036</td>
<td>(25.00 ± 0.055)</td>
</tr>
<tr>
<td>2.76 (6.06) ± 1.81</td>
<td>-108.67 ± 0.029</td>
<td>(24.97 ± 0.044)</td>
</tr>
</tbody>
</table>
Table 2: IVC, aorta and kidney temperatures (mean ± SD) for three mice measured over a 24 minute period using TmDOTMA- thermometry. Statistically significant differences between tissue compartments are indicated in bold (Student’s t-test).

<table>
<thead>
<tr>
<th></th>
<th>Temperature IVC (°C)</th>
<th>Temperature Aorta (°C)</th>
<th>Temperature Kidney (°C)</th>
<th>IVC vs Aorta (p values)</th>
<th>IVC vs Kidney (p values)</th>
<th>Aorta vs Kidney (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1</td>
<td>34.33 ± 0.26</td>
<td>34.12 ± 0.66</td>
<td>35.01 ± 0.46</td>
<td>0.4</td>
<td>0.003</td>
<td>0.007</td>
</tr>
<tr>
<td>Animal 2</td>
<td>32.28 ± 0.60</td>
<td>32.34 ± 0.31</td>
<td>33.16 ± 0.15</td>
<td>0.8</td>
<td>0.03</td>
<td>0.003</td>
</tr>
<tr>
<td>Animal 3</td>
<td>34.31 ± 0.54</td>
<td>34.00 ± 0.76</td>
<td>35.03 ± 0.33</td>
<td>0.3</td>
<td><strong>0.004</strong></td>
<td><strong>0.002</strong></td>
</tr>
</tbody>
</table>
Figure 1: Schematic of mechanical flow phantom used for temperature measurements for static and flowing TmDOTMA- (a). The phantom consists of two reservoirs which are connected by plastic tubing and a glass capillary tube positioned over a surface RF coil where MR signal is acquired. Flow through the system is produced by injecting air from a syringe pump into one of the reservoirs. Two fibre optic temperature probes are positioned within the flow circuit proximal and distal to the glass capillary tube. The apparatus sits in an insulated compartment within the bore of the scanner and is temperature regulated by circulating warmed air. The chemical shift difference between TmDOTMA- and water versus temperature for static (solid symbol) and flowing (open symbol) fluid ascertained using spectroscopic approach (b). Linear regression analysis shows a linear relationship for both static and flowing fluid ($R^2$ 0.9996 and 0.9980 respectively). 95% CI are indicated by the dashed line. No significant difference in the slope ($C_T$) was found (ANCOVA, p=0.56) for static and flowing fluid. A small difference in y-intercept ($C_o$) was found (ANCOVA, p=0.0028).
Figure 2: Temperature measurement of flowing fluid from phase evolution of TmDOTMA-. Flow velocity within 1 mm capillary tube was estimated using phase contrast methodology (mean velocity 2.5 cm/s, in plane resolution 0.2 x 0.2 mm) and shows a typical laminar flow profile with maximum velocity within the center of the capillary tube (a). Typical TmDOTMA-phase versus echo time for flowing fluid within the 1 mm channel (center voxel indicated by white arrows, in plane resolution 1 x 1 mm) illustrated with phase images multiplied by magnitude image (b). The corresponding TmDOTMA-phase evolution versus TE is plotted for static (solid symbol) and flowing fluid (open symbol) with linear regression and 95% CI (dashed line) shown (c).
Figure 3: Linear regression of temperature of flowing TmDOTMA- measured using phase methodology versus temperature measured using fibre optic probe showing good correlation between these two measurements ($R^2 0.9957$). Slope was close to unity ($1.02 \pm 0.019$) and intercept ($0.011 \pm 0.48$). 95% CI (dashed line) shown.
**Figure 4:** Axial images acquired through the mouse abdomen at the level of the right kidney. Water magnitude images with anatomical labels and ROIs for the temperature analysis are indicated (a). TmDOTMA- magnitude images with overlay of ROIs are shown (b). Slice thickness was 10 mm and the aorta and IVC are the only vessels that are visible in the field of view. Two reference tubes containing 10 mM TmDOTMA- are visible on the ventral aspect of the animal. Reference tube 2 did not extend across the entire imaging slice and differences in signal intensity are the result of partial volume averaging.

**Figure 5:** Temporal change in TmDOTMA- and water signal. The time scale represents approximate time after injection of TmDOTMA- contrast. The first time point for water signal (t=0 minutes) was acquired just prior to injection. Each data point is the mean SNR from three acquisitions with different TE and error bars indicate the standard deviation (σ). The TmDOTMA- signal is maximal in each compartment at the first time point (3 minutes) and subsequently decays (a). Water signal demonstrates an initial decrease resulting from
susceptibility related signal loss with eventual return of water signal to baseline as paramagnetic TmDOTMA- is cleared from each tissue (b).

**Figure 6:** Temporal measurement of temperature after bolus administration of TmDOTMA-. Temperature was ascertained in blood (aorta and IVC), kidney and reference tube. Each data point represents a single temperature measurement and the error bars are the standard deviation (σ) reflecting the uncertainty based on error in phase calculation. Mean temperature over 24 minutes in the aorta, IVC and kidney were 34.1°C (σ=0.7), 34.3°C (σ=0.3), and 35.0°C (σ=0.5) respectively. A significantly higher temperature was found for kidney compared to aorta (p=0.007, Student’s t-test) and IVC (p=0.003, Student’s t-test).