Mapping of Oxygen by Imaging Lipids Relaxation Enhancement: A Potential Sensitive Endogenous MRI Contrast to Map Variations in Tissue Oxygenation

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Purpose: Because of its paramagnetic properties, oxygen may act as an endogenous magnetic resonance imaging contrast agent by changing proton relaxation rates. Changes in tissue oxygen concentrations have been shown to produce changes in proton relaxation rate \( R_1 \) of water. The aim of the study was to improve the sensitivity of oxygen enhanced \( R_1 \) imaging by exploiting the higher solubility of oxygen in lipids (as compared with water) to sensitively monitor changes in tissue oxygen levels by selectively measuring the \( R_1 \) of lipids.

Methods: The method, with the acronym "MOBILE" (mapping of oxygen by imaging lipids relaxation enhancement), was applied in different mouse models of hypoxic processes on a 11.7 T magnetic resonance imaging system. MOBILE was compared with \( R_2^* \), \( R_1 \) of water, and with \( pO_2 \) measurements (using electron paramagnetic resonance oximetry). MOBILE was also applied in the brain of healthy human volunteers exposed to an oxygen breathing challenge on a 3 T magnetic resonance imaging system.

Results: MOBILE was shown to be able to monitor changes in oxygenation in tumor, peripheral, liver, and brain tissues. The clinical translation was demonstrated in human volunteers.


Key words: oxygen mapping; tissue hypoxia; MRI; \( R_1; \) endogenous contrast

There is a critical need for developing dynamic, noninvasive methods for oxygen mapping in clinical practice (1). Although hypoxia has been long recognized as a crucial factor in many disorders and in treatment, response/failure, atraumatic, ready-to-use, and reliable methods to quantify tissue oxygenation are still lacking in day-to-day clinical practice. Direct quantitative methods, including Eppendorf microelectrodes (2), electron paramagnetic resonance (EPR) oximetry (3,4), \( ^{19} \)F relaxometry (5), or Overhauser enhanced magnetic resonance imaging (MRI) (6), are either invasive or require the injection of a reporter probe, and are currently not clinically applicable. Nowadays, the clinically available armamentarium for this purpose only includes radiolabeled nitromidazoles detected by positron emission tomography (PET) which prominently accumulate in hypoxic areas, thereby acting as potential markers for tissue hypoxia (7,8). Endogenous sources of contrast in MRI include variations of \( T_1 \) (longitudinal relaxation rate) and \( T_2^* \) (effective transversal relaxation rate) values in tissue (9). \( T_1 \) discloses sensitivity to dissolved oxygen, which acts as a \( T_1 \)-shortening paramagnetic contrast agent (10). \( T_2^* \) is sensitive to the relative deoxyhemoglobin/oxyhemoglobin (Hb/HbO2) ratio in vessels (11) (Fig. 1a). \( T_2^* \) mapping, also referred to as functional MR imaging or blood oxygen level dependent (BOLD) imaging, is sensitive to variations in oxygenation in the vascular compartment, and has been successfully applied to monitor changes in tissue oxygenation (12–14). However, BOLD-MRI has also demonstrated significant limitations in terms of quantitative relationships between response signal intensity and true changes in tissue \( pO_2 \) and is sensitive to changes in total hemoglobin content (15–17). Recently, changes in tissue oxygen concentrations have been shown to produce changes in relaxation rate \( R_1 = 1/T_1 \) of water (18–20). Studies have demonstrated that oxygen-enhanced MRI produces measurable signal intensity changes in normal tissues using standard clinical MR systems. Thus
Scientific sensitivity and the measured level variations, in a different way than BOLD imaging. Unfortunately, the technique still suffers from insufficient sensitivity and the measured ΔR1 may be biased by confounds unrelated to changes in tissue oxygen level, such as alteration in blood flow, and tissue H2O content.

We hereby propose a method based on higher solubility of oxygen in lipids than in water (21). By monitoring changes in R1 relaxation rate of the lipid peak rather than those of water, estimates of variations in tissue oxygenation are obtained. We have developed an MR method for mapping variations in oxygenation based on changes in relaxation properties of the tissue lipids, which we called mapping of oxygen by imaging lipids relaxation enhancement (MOBILE). Since equilibration with 760 mmHg of O2 was demonstrated to have a significantly higher effect on lipid protons than on water protons (21), we hypothesized that the R1 of lipids should be more sensitive than the R1 of water to variations in tissue oxygenation (Fig. 1b). To assess the R1 value of lipids as a sensitive universal endogenous marker of tissue oxygenation, we recorded and analyzed changes in this parameter in hypoxic mouse models mimicking physiopathological conditions such as tumor hypoxia, peripheral ischemia, cerebral ischemic stroke, and liver steatosis, before and during a hyperoxic breathing challenge.

The choice of tumor models to assess the sensitivity of MOBILE to variations in oxygenation was done because tumor hypoxia is acknowledged as a major factor of resistance of solid tumors to treatment, in particular to external irradiation (22,23). Quantitative follow up of changes in tumor oxygenation can find relevant applications in radiation therapy planning (24–26) as well as with regard to antangiogenic and antivascular treatments optimization (27,28).

Peripheral vascular disease is responsible for the increased risk of lower limb amputation observed in diabetic patients and/or in patients presenting systemic atherosclerosis (29–31). A significantly better outcome is expected for these patients, based on improved technical options in peripheral revascularization (31,32). For that purpose, techniques able to assess quantitative peripheral PO2 are mandatory to verify the efficacy of therapeutic approaches aimed at salvaging limbs in diabetic patients with critical limb ischemia (CLI). In this context, we hypothesized that the MOBILE sequence could be a sensitive tool for assessing peripheral tissue oxygenation.

Mapping brain tissue oxygenation shortly after acute ischemic stroke may be crucial in selecting patients who may still benefit from thrombolytic treatment beyond conventional time-based guidelines (33,34). In this context, it seems mandatory to explore potential applications and limitations of oxygenation/perfusion mapping techniques in patient triage and patient eligibility for thrombolytic therapy in acute ischemic stroke (35). It is therefore useful to establish the potential relevance of MOBILE imaging in a stroke model as a complementary tool to the classic imaging protocol currently used to image acute or chronic strokes.

The assessment of liver oxygenation could be particularly important in the early monitoring of the liver after transplantation. Measurements of the global T1 before and after oxygen inhalation have been also reported recently as a quantitative imaging biomarker for the staging of liver cirrhosis (36). MOBILE was used to assess changes in liver tissue oxygenation, presenting abnormal

FIG. 1. MR oxygen-dependent endogen contrasts: principles of T2* and T1 contrasts. a: T2* (functional imaging): the contrast is based on the relative change in deoxyhemoglobin (Hb), which is paramagnetic; vs. oxyhemoglobin (HbO2), which is diamagnetic. Hb is able to decrease the effective transversal relaxation time T2* of protons, which will vary under different blood oxygen levels. Usually, R2* (1/T2*) is presented and is reported to be decreased with an increase in oxygenation. T1 (Oxygen enhanced longitudinal relaxation): the contrast is based on the paramagnetic properties of oxygen that induces a decrease in the longitudinal relaxation time T1 of tissue protons, which therefore varies under different tissue oxygenation levels. R1 (1/T1) is reported to be elevated with an increase in tissue oxygenation. b: MOBILE: the contrast is based on the change in the T1 of lipid protons instead of water protons. Indeed, as oxygen is more soluble in lipids than in water, the concentration of oxygen will be higher in a lipid phase than in a water phase for the same atmospheric pressure. In a tissue (which is composed of water and lipids), by sampling the T1 of lipid protons only and removing the signal coming from the water protons, a higher sensitivity to variations in tissue oxygenation can be achieved.
accumulation of lipids (steatosis being frequent in particular in obese and overweight patients) in response to a carbogen breathing challenge.

Finally, to assess the potential for translating MOBILE into clinical settings, we also report initial human data acquired from healthy volunteers breathing alternatively air and oxygen in a clinical 3 T MRI system.

METHODS

In Vitro Samples

Calibrations ($R_1$ as a function of percent oxygen) of the water and lipid peaks were performed by measuring $R_1$ at 37°C in sealed tubes containing water, sunflower oil, or tumor homogenates (excised MDA tumors homogenized with NaCl 0.9% solution, 1 mL/g) bubbled with nitrogen (0% O$_2$), air (21% O$_2$), or carbogen (95% O$_2$) for 20 min in a 37°C water bath. The temperature was controlled with OxyLite™ (Oxford Optronix, Oxford, UK) probe and kept constant using the warm water circulating system. The pH was monitored using a pH micro-electrode (Microelectrodes, Inc.).

Animal Models

Animals were anesthetized by inhalation of isoflurane (Forene, Abbot, England) mixed with 21% oxygen (air) in a continuous flow (1.5 L/h). This anesthesia has been shown not to interfere with tissue hemodynamic (37). Respiration rate and body temperature (37.0°C ± 1.0°C) were monitored and maintained with a circulating water blanket. Studies were undertaken in accordance with the national and local regulations of the ethical committee (agreement number LA 1230467). In vivo, four hypoxic mouse models mimicking physiopathological conditions such as tumor hypoxia, peripheral ischemia, and cerebral ischemic stroke, were used in this study.

Tumor Models

A total of 5 × 10⁶ NT2 cells (provided by E. Jaffee) or 3 × 10⁶ MDA-MB-231 (LGC Promochem), amplified in vitro, were collected by trypsinization, washed three times with Hanks balanced salt solution. These mammary tumor cells were injected subcutaneously into the right upper mammary fat pad of 6-week-old FVB/N or nude NMRI female mice (Janvier), respectively ($n = 5$ per model). Tumors were analyzed when reaching 6 mm in diameter.

Peripheral Ischemia Model

Femoral vein ligation was performed on C3H female mice 2 h before imaging. The femoral artery and vein were exposed and ligated with three sutures, one above and two after the origin of the natural collateral on the left hind leg of the mouse. Sutures were secured with surgical glue (Histoacryl; 3M). Three mice were studied (three ligated legs and three contralateral normal legs).

Stroke Model

Cortical photothrombosis was induced in six C57BL6/J mice (Jackson Laboratories; Bar Harbor, ME) 24 h before imaging according to (38). A vertical incision was made between the right orbit and the external auditory canal. The upper part of the temporalis muscle was cauterized so that the muscle could be displaced. Photoillumination with green light (wave length, 540 nm; bandwidth, 80 nm) was achieved using a Xenon lamp (model L-4887; Hamamatsu Photonics, Hamamatsu City, Japan) with heat-absorbing and green filters. The irradiation at intensity of 0.68 W/cm² was directed with a 3-mm optic fiber, which was placed on the exposed skin above the sensory motor cortex. Photoillumination was performed for 5 min after intravenous injection of the photosensitizer Rose Bengal (20 mg/kg, Sigma-Aldrich) in a tail vein.

Liver Model

Five ob/ob (B6.V-Lepob/J) leptin deficient C57Bl6 mice (Charles River, France) were used for the liver study.

Lipids Content

Total lipids were measured by gravimetry using an adaptation of Folch method (39). Lipids were extracted from brain and liver of healthy mice ($n = 3$), and from subcutaneous NT2 tumors ($n = 3$) in CHCl₃-MeOH (2:1) as previously described (40).

Preclinical Experiments

Three MR measurements of each type ($R_1$, H₂O, $R_1$ lipids, $R_2^*$) were acquired sequentially and repeated three times during air breathing. Then, breathing gas was switched to carbogen, and MR measurements were repeated at 10, 25, and 40 min after the gas switch. Respiratory triggering was employed to acquire images during the expiration cycle to avoid motion artifacts. In tumor experiments, we used the OxyLite™ fiber-optic microprobes for continuously monitoring tumor oxygenation simultaneously with MRI (41). Independent experiments were also performed to compare MOBILE with EPR Oximetry using the similar breathing challenge.

In vitro and animal experiments were performed with an 11.7 T (Bruker, Biospec), and with a quadrature volume coil (inner diameter of 40 mm).

In Vitro MR Data

The relaxation properties of two common lipid peaks (1.3 and 4.0 ppm) were exploited. The lipid peak with a chemical shift of ~1.3 ppm was ascribed to methylene protons from fatty acid chains and the peak with a chemical shift of ~4.0 ppm was ascribed to protons from the glycerol backbone of triglycerides (42–44).

$T_1$ Measurements

A segmented inversion-recovery fast imaging with steady state precession (IR FISP; 45) sequence (SSFP FID or Steady State Free Precession Free Induction Decay mode) was used to acquire parametric images of $T_1$ relaxation time (Fig. 2). The acquisition parameters were repetition time (TR)/TE/FA/BW/matrix = 4 ms/1.2 ms/5°/100 kHz/64 × 64, four segments, and a total
acquisition time of 1 min 20 s. For the total proton experiment (essentially reflecting the water peak), to sample the recovery of the signal, a series of 100 images were acquired (spaced by a scan TR = 120 ms to obtain images at different inversion times) with a slice thickness of 1 mm. For the lipid experiments, we first evaluated the difference in Hertz between water and lipid peaks in the 1H spectrum with a single pulse sequence. These offsets were then used as an imaging frequency offset in the same IR FISP protocol. Typically, the lipid peak of interest was $/C_{24}$ 4.0 ppm for tumor and brain tissues, and $/C_{24}$ 1.3 ppm for peripheral and liver tissue, as identified experimentally. We added a $\pi/2$ hermite saturation pulse with a bandwidth factor of 5400 Hz ms to spoil the water signal. A series of 40 images (spaced by scan TR = 100 ms) with a slice thickness of 3 mm were acquired. Then, images were fitted using a home-made program written in Matlab (The MathWorks, Inc., Natick, MA) to determine the $T_1$ relaxation in regions of interest (ROIs). For that purpose, a nonlinear fit was used to determine the $T_1$ relaxation in each pixel of the ROI. Raw data were filtered so as to disregard nonrelevant fits with a $T_1$ error/$T_1 > 30\%$. $T_1$ values were then calculated from the effective $T_1^*$ values measured experimentally (46–48): $M_0(t) = A = B\exp(-T_1/\tau)$, with $A = M_0(T_1^*/T_1)$, $B = M_0(1 + T_1^*/T_1)$, and $C = T_1^*$, and then $T_1 = (B/A - 1)$. $T_1^*$. Finally, $R_1 (1/T_1)$ data were filtered according to the calibration curves obtained from tissue extracts ($R_1$ 0% O$_2$ < measured $R_1 < R_1$ 100% O$_2$).

$T^*_{2}$ Measurements

For $T^*_{2}$ measurements, a multigradient echo sequence was performed with eight echoes (between 3.5 and 31.5 ms) with a total acquisition time of 4 min 48 s. A 256 x 256 matrix was used with TR/flip angle/slice thickness = 1500 ms/30°/1 mm.

**EPR Oximetry**

In vivo tumor pO$_2$ was monitored using EPR oximetry using charcoal as the oxygen-sensitive probe (49,50). EPR spectra were recorded using a 1.1 GHz EPR spectrometer (Magnettech, Berlin, Germany). Calibrations curves were made by measuring the EPR line width as a function of the pO$_2$. Mice were injected in the center of the tumor using the suspension of charcoal (100 mg/mL, 60 µL injected). The tumor under study was placed in the center of the extended loop resonator whose sensitive volume extends 1 cm into the tumor mass. The pO$_2$ measurements correspond to an average of pO$_2$ values in this volume of the NT2 tumor models. Charcoal was injected 24 h before experiments. MOBILE and EPR measures were performed the same day, and it was verified that charcoal did not perturb MOBILE or $T_1$ MRI measurements.

**Clinical Experiments**

MR imaging was performed at 3.0 T (Achieva; Philips Medical System) using a transmit/receive head coil. Three different imaging sequences were used during baseline acquisition (air breathing) and 5 min after initiation of oxygen (15 L/min). The oxygen was delivered by a mask placed by an elastic band around the subject’s head.

**$T_2$ Measurement**

A look locker sequence ($T_1$ TFE, $T_1$ turbo field echo sequence) was applied for 2 min with TR/TE/NA/flip...
angle/TFE = 3.467 ms/1.45 ms/1/5/10. A total of 140 images with 20 mm of thickness and 80 × 80 pixels were obtained. The lipid peak of interest in the brain of human volunteers was ~1.3 ppm, as identified experimentally.

**MOBILE Measurements**

The same sequence was used and a 90° SPIR pulse was added (spectral saturation by Inversion recovery) to spoil water with a BW of 300 Hz centered on the water peak. The acquisition lasted 4 min and 117 images with 20 mm of thickness and 80 × 80 pixels were obtained.

**T**<sub>2</sub> Measurements

A multiecho fast field echo sequence was performed with 15 echoes with a total acquisition time of 41.8 s. A 320 × 320 pixels matrix was obtained with TR/flip angle/slice thickness = 250 ms/18°/4 mm.

**Statistical Analysis**

Paired t-tests were used to compare mean changes between groups (carbogen or oxygen vs. air breathing; ischemic vs. control, R1 H2O vs. R1 lipids during carbogen challenge) for each parameter. Histogram distributions, linear fits, and Pearson correlation (with P values < 0.05 (*), <0.01 (**), or <0.001 (***)) were considered significant) were performed using the Graphpad software.

**RESULTS**

In Vitro Validation of MOBILE

To establish the sensitivity of R1 of lipids to variations in oxygenation, we assessed in vitro the relaxation properties of water and lipid components in pure aqueous and oil phases, in mixed systems, and in tissue homogenates, equilibrated in different oxygen environments (0%, 21%, and 95% O2). The higher solubility of oxygen led to a higher sensitivity when considering the evolution of R1 of lipids as a function of oxygenation, compared with the R1 of water, resulting in a higher sensitivity of R1 to oxygen measured in a lipophilic phase than in water (Fig. 3a–c). Typically, the gain in sensitivity (compared with water) corresponds to a factor of 7.5 in pure oil, 2.1 and 11.4 in tissue homogenates corresponding to 2 distinct common lipid peaks with a chemical shift of ~1.3 and ~4.0 ppm, respectively. The peaks were identified by comparing the liver and tumor tissue homogenate’s spectra with published chemical shifts of lipids (42–44), as well as with MR spectra of in vitro phantoms consisting of water/triglyceride (tripalmytelyglycerol) mixture. Inversion Recovery curves used to estimate the R1 parameter show the ability of the MOBILE sequence to suppress the water signal and selectively measure the lipid component of the signal on in vitro phantoms composed of pure water and oil (Fig. 3d–g).

Of note, the calibration curves were not significantly modified by changes in temperature or pH within the physiological range, namely, between 33 and 37°C and between 6.1 and 7.4 as pH values.

Sensitivity of MOBILE to Variations in Tumor Oxygenation

We used two mammary tumor models, syngeneic NT2 tumors and MDA human xenografts implanted orthotopically in mice. These models were selected for their large range of hypoxic fractions. We performed MR measurements before and during an hyperoxic breathing challenge protocol with carbogen. This gas induces significant transient increases in oxygenation in experimental and in some human tumors (12,13,51). The increase in oxygenation was demonstrated using a direct invasive technique simultaneously in the same tumors. Indeed, the measurements were simultaneously correlated with (i) a quantitative local measurement of tumor pO2 using invasive MR compatible fiber optic fluorescence quenching probes (OxyLite<sup>TM</sup>) (Fig. 4a), and (ii) two currently used MR parameters reflecting changes in tissue oxygenation, namely, R*<sub>2</sub> and R<sub>1</sub> of water. Fluorescence quenching measurements are direct and quantitative, enabling longitudinal follow-up studies performed simultaneously to MR measurements (52). The basal pO2 in MDA tumors ranged from 2 to 25 mmHg (mean of pO2 = 14.15 ± 5.46 mmHg) whereas basal pO2 in NT2 tumors was 37 ± 0.8 mmHg. Tumor oxygenation was increased in the majority of the tumors (7 of 8) under carbogen breathing conditions. A typical OxyLite graph shows the dynamic and the range of response for a typical tumor (Fig. 4b). After carbogen breathing, MDA and NT2 tumors reached a pO2 around 41.2 ± 2.3 and 87.7 ± 14.1 mmHg, respectively. Mean relative changes in relaxation rates with respect to the hyperoxic challenge were compared (pooled results for both tumor models) between R1 H2O, R1 lipids, and R*<sub>2</sub>, with relative changes of 21 ± 1.0% (P < 0.05; ∆R1 H2O), 10.1 ± 4.8 % (P < 0.05; ∆R1 lipids), and 9.8 ± 4.6 % (P < 0.05; ∆R*<sub>2</sub>) (Fig. 4c). Although values are rather dispersed, an increase by a factor of 4.8 was achieved for R1 lipids compared with R1 H2O. Typical maps of the relative difference in relaxation rates before and during the carbogen challenge were generated to compare the sensitivity of the R1 of lipids (MOBILE), R1 of H2O, and R*<sub>2</sub> methods (Fig. 4d–f), indicating that ∆R1 lipids are more sensitive than ∆R1 H2O on the same tumor. The corresponding histograms were generated to visualize and quantify the shift in the distributions for each parameter (Fig. 4g–i), showing a larger shift in the median value for ∆R1 lipids compared with ∆R1 H2O. In a parallel study, we compared EPR oximetry and R1 lipids and R1 H2O in the NT2 tumor model. The basal pO2 was 8.4 ± 1.1 mmHg and reached 26.1 ± 6.1 mmHg after carbogen breathing. The correlation between R1 H2O and pO2 was not significant (P = 0.8611, positive linear fit 0.000409 ± 0.002241; r<sup>2</sup> = 0.005529) (Fig. 5a), while a positive linear significant correlation was found between R<sub>1</sub> lipids and pO2 (0.01744 ± 0.00656; r<sup>2</sup> = 0.5407; P = 0.0376) (Fig. 5b).

Application of MOBILE in a Peripheral Ischemia Model

In the limb ischemia model, we tested the sensitivity of MOBILE to (i) basal peripheral tissue oxygenation by comparing R1 of lipids in tissues downstream the artery ligation and in the control leg, and (ii) relative changes...
during a carbogen breathing challenge. We hypothesized that the ischemic tissues may have less capacity to respond to the hyperoxic challenge than the control leg. In the management of CLI, it was suggested that a 10 min 100% oxygen challenge could be performed to determine the outcome of wound cicatrization, based on the absence or presence of response to the hyperoxic challenge (53). Some groups also focused in the past on hyperbaric oxygen therapy to improve the outcome of nonhealing wounds (54). Here, we show that $R_1$ lipids is more sensitive than $R_1$ H$_2$O and $R^*_2$ to evidence (i) differences in basal tissue oxygenation (control vs. ischemic leg) (Fig. 6a) and (ii) relative changes in response to carbogen breathing (Fig. 6b,c). Significant changes in mean values were observed in the control leg in response to carbogen breathing for $\Delta R_1$ lipids (7.4 ± 2.0%, $P < 0.05$), whereas no significant changes were observed for $\Delta R_1$ H$_2$O (1.3 ± 0.8%) or $\Delta R^*_2$ (9.6 ± 3.8%, $P > 0.05$) (Fig. 6b). A sensitivity increase by a factor of 5.7 was achieved for $\Delta R_1$ lipids compared with $\Delta R_1$ H$_2$O. As expected, a lack of response to carbogen breathing was observed in the ischemic leg (Fig. 6c). Typical maps of the relative difference in relaxation rates before and during the carbogen challenge were generated to compare the sensitivity of the $R_1$ lipids (MOBILE), $R_1$ H$_2$O, and $R^*_2$ methods (Fig. 6d). This confirms highest sensitivity for changes in $R_1$ lipids compared with $R_1$ H$_2$O and inhomogeneous changes in $R^*_2$.

Application of MOBILE in a Stroke Model

We used the photothermotic stroke model with unilateral lesion in mice to test the sensitivity of MOBILE to: (i) basal brain tissue oxygenation by comparing the intact

![Figure 3](image-url)
and the insulted hemispheres, and (ii) response to carbogen breathing in the intact (control area) and insulted (stroke area) hemispheres. We hypothesized that the stroke infarct would not be able to respond to a carbogen challenge as well as the control contralateral area. We demonstrated that MOBILE was able to identify differences in basal brain tissue oxygenation (Fig. 7a) but was also highly sensitive to normal brain tissue hyperoxygenation during the carbogen challenge (Fig. 7b), in contrast to the insulted area (Fig. 7c). The relative change in $R_1$ lipids was 4.5 times higher than the relative change in $R_1$ H$_2$O in response to carbogen breathing in the control area (Fig. 7b). The stroke area did not respond to carbogen breathing using MOBILE or BOLD MRI but showed a significant increase using $R_1$ H$_2$O (Fig. 7c).

Application of MOBILE in a Liver Steatosis Model
Liver of mice were imaged before and during a carbogen breathing challenge ($n = 10$). The sensitivities to changes in liver oxygenation were compared in terms of $R_1$ H$_2$O and $R_1$ lipids. We observed a significative higher sensitivity of $R_1$ lipids compared with the $R_1$ H$_2$O during the carbogen breathing challenge ($P = 0.023$) that resulted in increased tissue oxygenation (Fig. 8).
FIG. 5. Comparison of MOBILE with EPR to assess tumor oxygenation. a, b: Correlation between global pO2 measurements and global $R_1$ values (a) or lipids $R_1$ values (b).

FIG. 6. Application of MOBILE in a peripheral ischemia model. a: Basal relaxation times (global $R_1$ and $R_1$ lipids ± SEM) in control legs and in ischemic legs. Note the higher sensitivity of $R_1$ lipids (MOBILE) compared with global $R_1$ while comparing control and ischemic status. b: Pooled results of control legs ($n = 3$): relative changes in relaxation times under air and carbogen breathing conditions for global $R_1$ (water), $R_1$ of lipids, and $R^*_2$. Note the higher change in $R_1$ of lipids vs. global $R_1$ in response to carbogen breathing. c: Pooled results of ischemic legs ($n = 3$): relative changes in relaxation times under air and carbogen breathing conditions for global $R_1$ (water), $R_1$ of lipids, and $R^*_2$. Note the lack of significant change in all parameters after femoral artery ligation. d: Anatomical images of a typical control leg (up) and ischemic leg (bottom) and their corresponding maps of differences in relaxation times (global $R_1$, $R_1$ of lipids, and $R^*_2$).
Lipids Gravimetry Results

The total lipid content ranges from $60.6 \pm 0.2 \, \mu g/mg$ liver to $93.6 \pm 11.8 \, \mu g/mg$ brain. The mean value established in the NT2 tumors is $106.1 \pm 20.4 \, \mu g/mg$ tumor.

Application of MOBILE in Clinical Settings

The potential for clinical translation of the MOBILE sequence was assessed by: (i) implementing the sequence on a clinical 3 T MRI system; (ii) verifying appropriate water suppression and $T_1$ sampling of lipids on phantoms, and (iii): assessing $R_1 H_2O$, $R_1$ lipids, and $R^*_2$ in brains of healthy volunteers under air and oxygen breathing conditions. A similar axial-transverse slice location in the brain of three volunteers was imaged during air breathing and after 5 min of 100% oxygen breathing. Typical results are shown in Figure 9 where matched transversal brain maps of $R_1 H_2O$, $R_1$ lipids, and $R^*_2$ were used to assess the relative changes in relaxation times under air and carbogen breathing conditions.

FIG. 7. Application of MOBILE in a stroke model. a: Basal relaxation times ± SEM (global $R_1$ and $R_1$ lipids) in control ($n = 10$) and insulted (stroke) ($n = 6$) hemispheres. Only regions with more than 10 pixels were included in the data (nonrelevant fit with a $T_1$ error/$T_1 > 30\%$ were discarded). b: Pooled results of control hemispheres: relative changes in relaxation times under air and carbogen breathing conditions for global $R_1$ (water), $R_1$ of lipids, and $R^*_2$. Note the higher change in $R_1$ of lipids vs. global $R_1$ in response to carbogen breathing. c: Pooled results of “stroke” hemispheres: relative changes in relaxation times under air and carbogen breathing conditions for global $R_1$ (water), $R_1$ of lipids, and $R^*_2$. d: Typical anatomical image (transversal slice) of a mouse brain with the arrow pointing to the stroke area and the line delimitating the ROI surrounding the stroke area. e: Corresponding typical global $R_1$ map of the brain under air breathing conditions. f: Corresponding typical “$R_1$ lipids” map of the brain under air breathing conditions. g: Corresponding typical $R^*_2$ map of the brain under air breathing conditions.

FIG. 8. Application of MOBILE in a liver steatosis model. a: Mean relative changes over the entire liver in relaxation times under air and carbogen breathing conditions for global $R_1$ (water), $R_1$ of lipids, and $R^*_2$ [$R = 1/T$] ($n = 10$ per group). Note the higher change in $R_1$ of lipids vs. global $R_1$ in response to carbogen breathing. b: Typical $R_1$ lipids map in response to carbogen breathing.
$R_2^*$ along with the corresponding anatomical image are shown (Fig. 9a–d). The mean relative changes are shown in three arbitrary distinct ROIs in the left and right hemispheres, and in the center (Fig. 9e–g). A high heterogeneity of response is observed for all three parameters, as indicated by the maps and histograms of ROI 2 and of the right hemisphere (Fig. 9i–k,m–o). Nevertheless, we can observe more “positive responding regions” in the $\Delta R_1$ lipids map than in the $\Delta R_1$ H2O map. This is also illustrated in the histograms, where the median of the

FIG. 9. Application of MOBILE in the clinical setting on healthy volunteers. a,h,l: Anatomical image of a typical volunteer brain. Three arbitrary ROIs have been defined. b–d: Typical map of changes in relaxation times in response to 5 min 100% oxygen breathing ($\Delta R = R_{\text{carbogen}} - R_{\text{air}}$) for global $\Delta R_1$ (b), $\Delta R_1$ lipids (c), and $\Delta R_2^*$ (d). e–g: Mean relative changes in relaxation rates (global $R_1$, water, $R_1$ of lipids, and $R_2^*$) in ROI 1 (e), ROI 2 (f), and ROI 3 (g). Note the systematic higher sensitivity of $R_1$ of lipids vs. global $R_1$. i–k: Corresponding histograms (of relative changes in relaxation rates) for ROI 2. Note the clear shift to the right of the median of $\Delta R_1$ lipids whereas the change in $\Delta R_1$ of water is slightly negative (not responding to oxygen breathing). The change in $\Delta R_2^*$ is slightly negative, as expected (responding to oxygen breathing). m–o: Histograms of relative changes in relaxation rates for the right hemisphere. Note the clear shift to the right of the median of $\Delta R_1$ lipids whereas the change in $\Delta R_1$ of water is slightly negative (not responding to oxygen breathing). The change in $\Delta R_2^*$ is slightly positive (not responding to oxygen breathing).
\[ \Delta R_1 \] lipids is shifted to the right (vs. the “zero value” bin). We showed that both MOBILE (supposed to respond positively to an increase in tissue oxygenation) and BOLD \( (R^*_{2}) \) imaging (supposed to respond negatively to an increase in blood oxygenation) are more sensitive to the changes in oxygenation than \( R_1 \) \( H_2O \). Similar data were obtained on two additional volunteers. Our first clinical results suggest that MOBILE using changes of \( R_1 \) lipids and BOLD are complementary in nature with a higher sensitivity than \( R_1 \) \( H_2O \).

**DISCUSSION**

Our goal was to implement and validate a new MR technique using an endogenous contrast to sensitively detect and map variations in tissue oxygenation. Starting from the previously described phenomenon of oxygen-enhanced relaxation of water protons \( (T_1) \), we developed a new measurement method based on the relaxation of lipid protons with the knowledge that oxygen is six times more soluble in lipophilic media than in water. Using the MOBILE sequence, we achieved (i) the development of an MR pulse sequence allowing longitudinal sampling of the relaxation time of lipid protons, and without any contamination from water signal; (ii) in vivo validation of the method on tissues undergoing variations in oxygenation, by comparing results from the new technique to those obtained by the standard \( T_1 \) \( H_2O \) and “BOLD” methods as reference; and (iii) comparison with direct quantitative reference methods (OxyLite™ and EPR) in the mammary tumor models.

We demonstrate that MOBILE is sensitive to changes in tissue oxygenation. The mammary tumor, peripheral ischemia, and cerebral stroke models were subjected to a hyperoxic breathing challenge using carbogen, whose response may be an index of the vascular functionality of those tissues. MOBILE demonstrates sensitivity to discriminating between vascularly dysfunctional tissues (tumor areas, ischemic limbs, or stroke) and normofunctional ones (i.e., contralateral leg or brain hemisphere). MOBILE is also sensitive to the baseline tissue oxygenation when comparing diseased with healthy tissues (in the peripheral ischemic and cerebral stroke models). Correlations between data sets from MOBILE and direct \( O_2 \) measurements demonstrated that \( R_1 \) lipids was significantly correlated with \( pO_2 \) measured by EPR. Nevertheless, we should bear in mind that \( R^*_{2} \) contrast arises primarily from hemodynamic responses whereas \( R_1 \) is likely to be mainly related to tissue oxygenation levels (Fig. 1). Therefore, information provided by the two relaxation parameters should be complementary.

This proof-of-concept study encompasses some potential applications of the MOBILE technique, by showing consistency of semiquantitative data (relative changes in \( R_1 \) lipids in response to an induced change in oxygenation in different tissues), although further crossvalidations with other quantitative in vivo or ex vivo techniques should be warranted to further assess the robustness of quantitative data yielded by the method. Nevertheless, MOBILE presents the advantage of allowing longitudinal studies, contrary to positron emission tomography imaging. Yet, the hereby presented models of peripheral ischemia and stroke which enable comparison between damaged areas and normal contralateral ones unequivocally demonstrate significant and constant differences of the basal \( R_1 \) lipids within the two areas. Also, oxygen enriched gases inhalation was used as a technique to modulate tissue oxygenation. Yet, actual effects of such gases might be variable from one tissue to the other e.g. according to the presence or absence of \( CO_2 \) (i.e. for carbogen). This may explain the dispersion of the results, particularly in tumors where tissue heterogeneity is high. Local \( pO_2 \) was assessed quantitatively using EPR and OxyLite in tumors to address this limiting concern. Interestingly, the short acquisition time of the MOBILE sequence should allow the follow-up of acute hypoxia in tumors in a quantitative and dynamic way, similarly to previous work using \( ^{19}F \)-relaxometry or EPR oximetry (53,56).

From a technical point of view, a current limitation of the technique is that the minimal amount of lipids required within a tissue to be enable accurate measurements using the MOBILE technique yet remains to be determined, although consistent data were obtained from such different tissues as malignant tumors, liver, muscle, and brain. This would also depend on extrinsic parameters such as the magnetic field strength and coils and could be a limitation to the application of the technique to some body’s areas. Also, at 11.7 T, two different lipid peaks were considered depending on the tissue of interest (at ~1.2 and ~4.0 ppm). This was established experimentally with respect to the peaks that could be reliably fitted in each tissue and was defined as an “operational protocol.” Nevertheless, at lower magnetic fields (i.e. in the clinical setting), only the 1.2-ppm peak is amenable for analysis. Finally, it would be relevant to compare the SSFP method used in this study to assess \( T_1 \) of water and lipids with \( T_1 \) fits issued from fat and water images extracted using the Dixon method or the alternative “IDEAL” method (57). Indeed, the use of a saturation pulse of the water peak might affect the lipid peak at 4.0 ppm and the resulting \( T_1 \) fit, although in vitro validation on phantoms shows that the SSFP method allows recovering reliable \( T_1 \) of lipids. Yet, the in vivo comparison of the methods would be required to select the best sequence to assess proper \( T_1 \) lipids values in vivo, especially since the maps of \( R_1 \) lipids sometimes show poor resolutions in some tissues because of the exclusion of some pixels in the postprocessing of the data (i.e., pixels with high \( T_1 \) fit errors).

Endogenous contrast arising from variations in lipids \( R_1 \) relaxation under different oxygenation conditions could find straightforward applications in any lipid containing tissue and could be readily translated into patient management on clinical MR scanners, without special requirements for software/hardware (i.e., dedicated coil systems, etc.). The technique is noninvasive, more sensitive than that using \( T_1 \) of the water signal, and should not be influenced by other hemodynamic factors, unlike \( T_1 \) \( H_2O \) or \( T^*_{2} \). The spatial and temporal resolutions (~0.6 mm, acquisition time 1 min 20 s) of the technique are relevant for dynamic mapping of oxygenation in both research (small animals) and clinical frames. Potential applications of the technique could be...
found in any pathophysiological condition where oxygen plays a key role, such as tumor oxygenation, cerebral stroke, peripheral ischemic diseases consecutive to, e.g., diabetes, or liver transplantation, as illustrated by the present work. Another possible field of application is the change in tissue oxygenation after physiological stimulation. An initial translational study on a clinical MR scanner was successfully performed on three healthy volunteers with spatial and temporal resolutions of \(-3.0\) mm and 4 min, respectively, thereby assessing a wide range of potential applications of the technique in clinical practice.

In conclusion, these initial studies suggest that MOBILE has the potential to provide a noninvasive and quantitative method for obtaining fast and robust measurements of the variations in tissue oxygenation in different pathological conditions, with a unique translational aspect “from laboratory to patient’s bed.”

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