In vivo mapping of deep tissue pO2 in a murine model of peripheral artery disease by noninvasive ^19F MR relaxometry

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RESEARCH LETTER

In Vivo Mapping of Deep Tissue pO_2 in a Murine Model of Peripheral Artery Disease by Noninvasive ^{19}F MR Relaxometry

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Peripheral arterial disease is characterized by varying degrees of hypoxia due to chronic progredient occlusion of peripheral arteries. Here, we report an in vivo approach for direct determination of tissue pO_2 in peripheral arterial disease with background-free, noninvasive fluorine magnetic resonance imaging (^{19}F MRI) using physiologically inert perfluorocarbon nanoemulsions.

Perfluorocarbon nanoemulsions dissolve paramagnetic oxygen proportional to the ambient pO_2, resulting a linear increase in the ^{19}F relaxation rate R_1 (R_1=1/T_1).[1] However, for pO_2 calculation, the temperature dependence of ^{19}F T_1 relaxation has to be considered and a fast, artifact-free magnetic resonance imaging acquisition technique is required. For this, FAIR-EPI (flow-sensitive alternating inversion-recovery echo planar imaging sequence) was used at 9.4T (Bruker AVANCE^{NEO}) with a 25-mm resonator tunable to both ^1H (linear) and ^{19}F (quadrature). Rescaling of the trajectories acquired in reference ^1H flow-sensitive alternating inversion-recovery echo planar imaging sequence scans allowed the elimination of ghosting artifacts along the phase encoding direction for the corresponding ^{19}F measurements (Figure [A]). Calibration curves were recorded within 31 °C to 39 °C using a 20% perfluoro-15-crown-5 ether nanoemulsion[2] utilizing the setup in Figure [B]. As expected, we found a temperature-dependent linear relationship between ^{19}F relaxation rate R_1 and pO_2 (Figure [C]).

Reference values were determined within thigh/calf of anesthetized mice by invasive sensors (NTH-PSt7/OXY-4; MIT-18/testo-108), yielding pO_2 of 26.2±2.0 mmHg (n=5) and temperatures of 34.6±0.4/33.8±0.2 °C for thigh/calf (n=5), the latter were used for conversion of in vivo ^{19}F R_1 to corresponding pO_2 using the formula in Figure [C].

Thereafter, ^{19}F MRI was applied to assess tissue pO_2 in a murine hindlimb ischemia model (HLI; see Figure [D], left; male 16–18 weeks C57BL/6J mice as approved by local authorities [LANUV]).[3] For determination of tissue pO_2, directly after surgery, 100 µL perfluorocarbon nanoemulsions were injected into the muscle of both upper hindlimbs (n=9) just below the first site of ligation and in a subset of n=3 animals also 50 µL perfluorocarbon nanoemulsions into both calf muscles (Figure [D], arrows). After 24 hours, ^{19}F T1 was determined to quantify pO_2 in both hindlimbs. While pO_2 in sham thighs was found to be 28.8±6.8 mmHg (the same range as reference values above), pO_2 in ischemic legs was significantly decreased to 11.6±4.6 mmHg (Figure [D] and [E], n=9 each; P=2.2×10^{-5}) as validated by invasive measurements (13.8±4.8 mmHg at unchanged temperatures of 34.6±0.3 °C). The calf revealed similar pO_2 at the control site, but an even larger pO_2 drop in HLI (31.2±6.4 versus 4.2±3.3 mmHg, n=3 each; P=0.006).

Taken together, ^{19}F MRI (1) resulted in similar pO_2 values as invasive measures and allowed (2) the assessment of the degree of tissue hypoxia as well as (3) mapping of the gradual pO_2 profile toward the periphery associated with the utilized peripheral arterial disease model. Beyond current perfusion/optical techniques or superficial O_2 measurements, our approach allows assessment of deep tissue pO_2, providing a meaningful

Key Words: echo-planar imaging ■ fluorine ■ hypoxia ■ magnetic resonance imaging ■ mitral valve insufficiency ■ peripheral arterial disease

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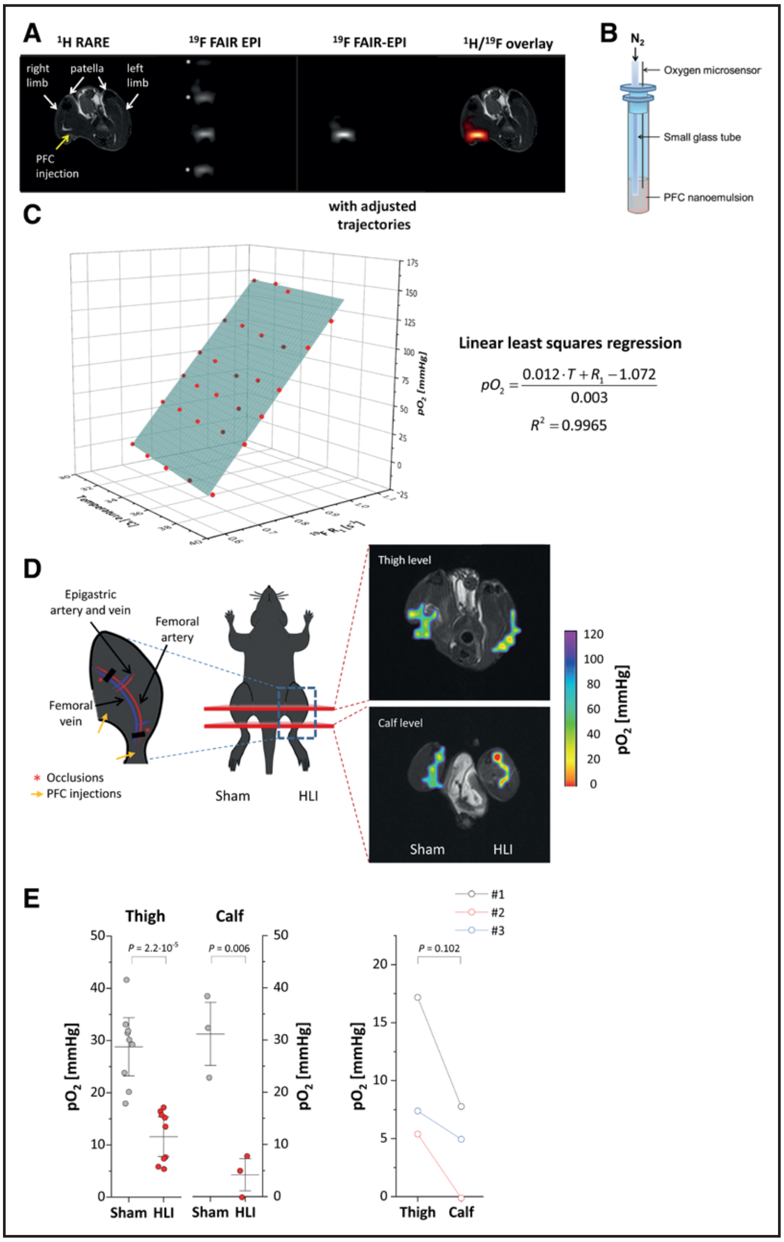

Figure. ^{19}F MRI relaxometry reveals gradual tissue pO_2 profiles in murine hindlimb ischemia (HLI).
A, For in vivo validation of the pulse sequence, PFCs were injected into the right murine hindlimb (yellow arrow). First: ^1H anatomical reference (RARE), Second: FAIR-EPI (flow-sensitive alternating inversion-recovery echo planar imaging sequence) ^{19}F image with ghosting artefacts (asterisks), Third: artifact-free FAIR EPI ^{19}F image with adjusted trajectories, Fourth: merge of ^{19}F (hot iron) and ^1H greyscale images. ^{19}F FAIR-EPI: T_E: 8.72 ms, T_R: 5000 ms, ST: 2 mm, matrix: 64×64, FOV: 40×40 mm^2, 48, T_I: 25/400/800/1200/1600 ms; T_{Acq}: 20 minutes). **B**, Calibration setup for pO_2 determination parallel to acquisition of T_1 maps, starting at ambient pO_2 and gradually displacing oxygen by step-wise flushing with nitrogen. pO_2 was continuously monitored with a fiber optic pO_2 probe. **C**, Fitted surface from the calibration curves acquired at 31/33/35/37/39 °C (n=3). **D**, Scheme of the HLI model (zoom-in left) and slice localization (red, middle) to acquire spatially resolved pO_2 maps for upper/lower limbs (right). **E**, Left: pO_2 for sham vs HLI for thigh/calf. pO_2 was calculated from measured ^{19}F R_1 with the formula in **C** using 34.6/33.8 °C for thigh/calf as obtained from invasive measurements. Right: pO_2 in ischemic thigh/calf for those animals receiving PFC injections in both regions. A Student *t*-test was utilized to determine statistically significance (*P* value <0.05).

measure of the consequences of impaired perfusion that reflects the true mismatch between demand and supply of the affected tissue. With this, our technology implies a substantial translational potential for early diagnosis of limited tissue supply.

ARTICLE INFORMATION

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Disclosures

None.

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