Quantitative and simultaneous measurement of oxygen consumption rates in rat brain and skeletal muscle using $^{17}$O MRS imaging at 16.4T

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Purpose: Oxygen-17 ($^{17}$O) MRS imaging, successfully used in the brain, is extended by imaging the oxygen metabolic rate in the resting skeletal muscle and used to determine the total whole-body oxygen metabolic rate in the rat.

Methods: During and after inhalations of $^{17}$O$_2$ gas, dynamic $^{17}$O MRSI was performed in rats ($n=8$) ventilated with N$_2$O or N$_2$ at 16.4 T. Time courses of the H$_2^{17}$O concentration from regions of interest located in brain and muscle tissue were examined and used to fit an animal-adapted 3-phase metabolic model of oxygen consumption. CBF was determined with an independent washout method. Finally, body oxygen metabolic rate was calculated using a global steady-state approach.

Results: Cerebral metabolic rate of oxygen consumption was $1.97 \pm 0.19 \mu$mol/g/min on average. The resting metabolic rate of oxygen consumption in skeletal muscle was $0.32 \pm 0.12 \mu$mol/g/min and >6 times lower than cerebral metabolic rate of oxygen consumption. Global oxygen consumed by the body was $24.2 \pm 3.6$ mL O$_2$/kg body weight/min. CBF was estimated to be $0.28 \pm 0.02$ mL/g/min and $0.34 \pm 0.06$ mL/g/min for the N$_2$ and N$_2$O ventilation condition, respectively.

Conclusion: We have evaluated the feasibility of $^{17}$O MRSI for imaging and quantifying the oxygen consumption rate in low metabolizing organs such as the skeletal muscle at rest. Additionally, we have shown that CBF is slightly increased in the case of ventilation with N$_2$O. We expect this study to be beneficial to the
application of $^{17}$O MRSI to a wider range of organs, although further validation is advised.

**KEYWORDS**

cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO$_2$), mitochondrial water, muscle resting metabolic rate of oxygen consumption, $^{13}$O MRSI of H$_2^{17}$O, skeletal muscle metabolism, total body oxygen consumption (VO$_2$)

1 | INTRODUCTION

Noninvasively measuring cellular oxygen metabolism using oxygen-17 ($^{17}$O) tracer and in vivo $^{17}$O spectroscopic imaging ($^{17}$O MRSI) at ultrahigh field is a promising tool for studying cellular energy metabolism and physiology. The $^{17}$O imaging approach allows to quantify the cerebral metabolic rate of oxygen (CMRO$_2$) in human and animal brain. Although imaging studies have been performed to differentiate the cerebral metabolic rates between gray and white matter with $^{17}$O, few measurements were done outside the brain, and they often focused on aerobic organs with a high metabolic rate. Obviously, lower metabolic rates, such as in resting muscle tissue, result in slower turnover rates from $^{17}$O$_2$ to H$_2^{17}$O in the mitochondria. Thus, less labeled H$_2^{17}$O signal in tissue could necessitate longer scans or require more application of $^{17}$O-isotope labeled O$_2$ to achieve an adequate SNR for imaging. The dynamics of H$_2^{17}$O signal change as well as an uncertainty in modeling with resulting longer inhalation durations is affected by blood perfusion and recirculation. This unmet challenge motivates our investigation of the feasibility of imaging the low oxygen metabolic rate in resting skeletal muscle using $^{17}$O MRSI in simultaneous comparison to the brain oxygen metabolism rates in the same subject. Using significantly longer $^{17}$O$_2$ inhalation times, the amount of generated H$_2^{17}$O in biological tissues and the resulting $^{17}$O MR signal are largely increased, even more so by multiple inhalations, allowing to reliably observe metabolic and perfusion parameters at increased sensitivity. However, in advantage against $^{15}$O PET, no subtraction scans have to be performed in $^{17}$O imaging for the exclusion of vascular or gaseous oxygen signal.

In this study, we simultaneously acquired dynamic time courses of H$_2^{17}$O signals in brain and muscle tissue in rats during $^{17}$O$_2$ inhalations using 3D $^{17}$O MRSI at an ultrahigh magnetic field of 16.4 T. Repetitive and longer inhalations of $^{17}$O$_2$ gas than in previous measurements in rodents resulted in a large increase of the H$_2^{17}$O concentration several times above natural abundance. The H$_2^{17}$O dynamic signals were fitted with 2 commonly used models: (1) a 3-phase metabolic model using the entire H$_2^{17}$O dynamic time course acquired before, during, and after inhalations to determine the metabolic rates of oxygen consumption; and (2) a washout model applied to the postinhalation brain data to estimate CBF. The 3-phase metabolic model, which was previously used in human gray and white matter, was modified to obtain the low resting-state metabolic rate of oxygen consumption in the rat skeletal muscle (muscle RMRO$_2$) for exploring the feasibility of using the $^{17}$O MRSI method in other tissues. The washout technique, allowing for estimation of CBF as previously validated in rodent brain, was used to investigate 2 groups of rats ventilated with different blends of gases (oxygen with N$_2$ or N$_2$O). Finally, recirculation of H$_2^{17}$O leading to a new equilibrium at the end of the postinhalation period was observed and employed to estimate the organism’s global metabolism rate (ie, total body oxygen consumption (VO$_2$)), which was then compared to the regional metabolic rates of oxygen consumption.

2 | THEORY

2.1 | Three-phase model adaptation

A previously published model for determining the human brain oxygen metabolism rate, fitting 3 phases of a H$_2^{17}$O time course (phase 1: before $^{17}$O$_2$ inhalation, phase 2: during $^{17}$O$_2$ inhalation, and phase 3: after $^{17}$O$_2$ inhalation), was adapted to the rat systemic characteristics. We chose this model for the study in rodents because it applies well to low metabolic rates involving a significant amount of recirculating H$_2^{17}$O. In particular with longer inhalations, cardiopulmonary factors such as the cardiac output are increasingly important.

The time-dependent brain tissue H$_2^{17}$O concentration defined as molar volume $M_v^{H_{17}O}(t)$ in an imaging voxel can be described as (refer to Equation (2) in Ref. 2 for more details):

$$\frac{dM_v^{H_{17}O}(t)}{dt} = 2 \cdot \text{CMRO}_2 \cdot A^{H_{17}O}(t) - K_L \cdot M_v^{H_{17}O}(t) + K_G \cdot B^{H_{17}O}(t).$$  

The 3 terms on the right side of Equation (1) can be separated into: (1) the regional metabolic activity producing...
H$_2^{17}$O (ie, the cerebral metabolic rate of oxygen consumption: CMRO$_2$), depending on the arterial $^{17}$O-isotope enrichment [$A^{17}$O$^2$] of oxygen gas delivered through hemoglobin; (2) the loss [$K_{Loss}$ or $K_L$] of H$_2^{17}$O mainly due to (cerebral) blood flow or perfusion washout into the draining venous vascularity; and (3) the gain [$K_{Gain}$ or $K_G$] of H$_2^{17}$O through inflow of blood [$B^{17}$O$^2$] containing H$_2^{17}$O, recirculating from both local metabolizing tissue and whole body oxygen metabolism.

By integration over time, Equation (1) can be used to fit the time courses (see Equation (6) in Ref. 2) of H$_2^{17}$O signal for each imaging voxel to derive the oxygen metabolic rate (MRO$_2$) in brain or muscle tissue (as CMRO$_2$ or RMRO$_2$). We propose herein that, in principle, for any sufficiently perfused organ, oxygen consumption rates even below the systemic global aerobic rate (VO$_2$) can be measured. The quantification is simplified if the water content of the imaged tissue, which can be calibrated by the H$_2$$^{17}$O natural abundance concentration and the $^{17}$O signal measured in phase 1, is known.$^{3,5}$ The water content of muscle and brain can be approximated by assuming comparability to humans (ie, mice$^{17}$: 74.4% weight [wt] in muscle vs. human$^{18}$: 79.5% wt in striated muscle and 73.3% wt in brain). Furthermore, the tissue density for rodents (1.06 kg/L for skeletal muscle$^{19}$) was employed for unit conversion. Equation (1) can then be used to determine the oxygen metabolic rates of the rodent muscle and brain.

### 2.2 Systemic oxygen expenditure VO$_2$

The total body oxygen consumption (VO$_2$ in the unit of μmol/g body weight/min) can be defined as the cumulative amount of metabolic H$_2^{17}$O added to the organism by inhalation and metabolism of $^{17}$O$_2$ tracer with a fixed enrichment within a given inhalation time. The average body oxygen metabolic rate (VO$_2$,average) per min can then be determined using the equilibrium H$_2^{17}$O signal in tissue measured during the late part of the postinhalation period (phase 3), assuming that a new equilibrium (or steady state of the tissue H$_2^{17}$O signal) has been established:

$$VO_2 = \frac{M_{V}^{H_2^{17}O} (equilibrium)}{t_{inhalation} \times f} = VO_2,average / f \ (t \gg t_{inhalation}) .$$

(2)

$t_{inhalation}$ is the inhalation duration; $M_{V}^{H_2^{17}O} (equilibrium)$ is the average tissue H$_2^{17}$O concentration ($M_{V}^{H_2^{17}O}$) at equilibrium (in this study, ~3 inhalation durations after $^{17}$O$_2$ inhalation) when its pre-inhalation level is set to 0; and the conventional format of VO$_2$ in volume of oxygen gas is usually given in mL/kg body weight/min and requires a unit conversion $f$ by division of 0.0446 μmol$^{-1}$mL$^{-1}$.

### 3 METHODS

#### 3.1 Simulation of circulation impact and metabolic rate on the H$_2^{17}$O time courses

Inhalations with a $^{17}$O$_2$ enrichment of 70% were simulated using the previously outlined 3-phase model for 2 settings: (1) simulation with a fixed high metabolic rate (ie, isometabolic CMRO$_2$ = 2 μmol/g/min) varying only the circulatory parameters ($K_G$ and $K_L$) in ranges reported in the literature$^{2,6,7,9,21}$ (phase 2 with 15.25 min inhalation duration); and (2) simulation of varying metabolic rate and corresponding changes in perfusion. In the second-stage simulation, different levels of local oxygen metabolic rates were set (MRO$_2$ = 2; 1; 0.5; 0 μmol/g/min) with fixed parameters $K_L$ = 0.2 and $K_G$ = 0.3, unless otherwise noted, during an inhalation using Equation (1) to qualitatively assess the time dependence of the tissue H$_2^{17}$O signal. Specifically, this allowed investigation under idealized conditions of the transitions between phases of the model.$^2$

Furthermore, we adapted and evaluated the rodent specific systemic recirculation parameter as detailed in the Supporting Information Figure S1.

#### 3.2 Animal preparation and physiology monitoring

All procedures and experiments were approved by the local authorities (Regierungsspräsidium [German for government council], Tübingen, Germany) and were in compliance with the guidelines of the European Community (EUVD 86/609/EEC) for the care and use of laboratory animals. A total of 8 male Wistar rats (Charles River Laboratories, Sulzfeld, Germany) were used in this study (Table 1) (mean body weight 312 ± 93 g). Artificial ventilation and maintenance of physiological stability is described in detail in the Supporting Information.

Ventilation mixtures with enriched $^{17}$O$_2$ gas (oxygen gas fraction ~25%-35% with 70% enriched $^{17}$O$_2$ Nukem GmbH, Germany) were prepared in nondiffusive gas bags (Hans Rudolph, Inc., Shawnee KS). Oxygen was mixed with N$_2$ in 1 group (Table 1, animals A-D; $n = 4$) and with N$_2$O in a second group of animals (Table 1, animals E-H; $n = 4$). At the end of each experiment, the animals were euthanized followed by postmortem imaging as previously reported.$^{22}$

#### 3.3 MRI instrumentation and data acquisition

MRI was performed on a BioSpec Avance III system (Bruker Biospin MRI GmbH, Ettlingen, Germany) using a 26 cm bore 16.4 T magnet and gradients with 12-cm inner diameter,
1 T m\(^{-1}\) maximum strength, and 212 μs ramp time (Resonance Research Inc., Billerica, MA). Custom-built quadrature surface coils (elliptical loops each ~1.5 × 1.2 cm) were tuned to the \(^{17}\text{O}\) Larmor-frequency (94.6 MHz) for \(^{17}\text{O}\) imaging, and a separate \(^1\text{H}\) butterfly RF coil passively decoupled from the \(^{17}\text{O}\) coils was used. Anatomical \(^1\text{H}\) MRI FLASH images with TR = 2 s, TE = 10 ms (nt = 4 averages), 59 × 59 μm\(^2\) in-plane resolution, and 29 axial slices (thickness = 1 mm) were acquired within 25 min 36 s.

A k-space acquisition-weighted 3D CSI pulse sequence was used for all \(^{17}\text{O}\) MRSI acquisitions. Two types of time series were acquired for each rat: natural abundance tissue \(^2\text{H}\)\(^{17}\text{O}\) signal before any \(^{17}\text{O}_2\) gas inhalation for calibration of \(^2\text{H}\)\(^{17}\text{O}\) concentration in each CSI voxel, and during and after a single or repeated inhalation for metabolic rate and CBF analysis. In all in vivo \(^{17}\text{O}\) MRSI acquisitions, we used a FOV of 27.5 × 12.5 × 18 mm\(^3\), spectroscopic sampling points 375, and acquisition duration of 3.75 ms with a delay of 0.538 ms from an excitation RF pulse. TR was 4.92 ms, optimized for tissue \(T_2^*\)\(^{-1}\), and RF-excitation was performed with a 68° hard pulse of 200 μs duration.

In the majority of animals (Table 1, animals A-F, referred to as high-resolution protocol), the FOV was scanned by an acquisition matrix of 15 × 7 × 7, resulting in a voxel volume of 43.1 μL as defined by the width of the spatial response function.\(^{24-26}\) Each 3D \(^{17}\text{O}\) CSI volume was acquired within 30.2 s, with a maximum number of averages \(n_{\text{max}} = 74\) at the k-space center (a total of 6144 FIDs or 735 k-space points per CSI volume). Fifty natural abundance \(^2\text{H}\)\(^{17}\text{O}\) CSI were acquired within ~25 min 36 s, and the same acquisition duration of 54 min 57 s was used to acquire 327 volumes of inhalation data. Other acquisition parameters remained the same.

Postmortem CSI-acquisitions were performed without k-space weighting (12 ms TR and 70° flip angle) and with a pulse length of 400 μs. A FOV of 27.5 × 12.5 × 25 mm\(^3\) was sampled with a matrix of 41 × 19 × 25 voxels (nominal voxel size 0.44 μL). Approximately a total of 2.5 million FIDs, with 1000 points each and a spectral bandwidth of 100 kHz, were acquired in 8 h 18 min.

### 3.4 Brain coregistration and tissue selection

The 3D \(^{17}\text{O}\)-CSI data were coregistered with \(^1\text{H}\) anatomic images and high-resolution (postmortem) \(^2\text{H}\)\(^{17}\text{O}\) images with the same FOV as illustrated in Figure 1A-C. Equally sized regions of interest (ROI) were selected (~3 mm Bregma)\(^{27}\) for brain and in lateral muscle compartments in the same coronal

| Individual Animal | Gas Mixture | Body Weight [g] | Inh. Number | Inh. Duration [min] |
|-------------------|-------------|-----------------|-------------|---------------------|
| Rat A             | \(^2\text{H}\) | 300             | 1           | 15.3                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.3                |
| Rat B             | \(^2\text{H}\) | 232             | 1           | 15.3                |
| Rat C             | \(^2\text{H}\) | 275             | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.1                |
| Rat D             | \(^2\text{H}\) | 233             | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 12.9                |
| Rat E             | \(^{2}\text{H}\)\(^{17}\text{O}\) | 250            | 1           | 15.4                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 3               | 1           | 15.1                |
| Rat F             | \(^{2}\text{H}\)\(^{17}\text{O}\) | 510            | 1           | 15.1                |
| Rat G\*           | \(^{2}\text{H}\)\(^{17}\text{O}\) | 332            | 1           | 15                  |
| Rat H\*           | \(^{2}\text{H}\)\(^{17}\text{O}\) | 367            | 1           | 15                  |

Each row represents 1 resting \(^{17}\text{O}_2\) inhalation measurement, which for rats A, C, D, and E was repeated multiple times within the same experimental session per animal.

\(^{17}\text{O}\), oxygen-17; Inh, inhalation.

**TABLE 1** Summary of performed inhalation numbers and inhalation durations and weight for each animal

| Individual Animal | Gas Mixture | Body Weight [g] | Inh. Number | Inh. Duration [min] |
|-------------------|-------------|-----------------|-------------|---------------------|
| Rat A             | \(^2\text{H}\) | 300             | 1           | 15.3                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.3                |
| Rat B             | \(^2\text{H}\) | 232             | 1           | 15.3                |
| Rat C             | \(^2\text{H}\) | 275             | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.1                |
| Rat D             | \(^2\text{H}\) | 233             | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 12.9                |
| Rat E             | \(^{2}\text{H}\)\(^{17}\text{O}\) | 250            | 1           | 15.4                |
|                   | \(^{17}\text{O}\) | 2               | 1           | 15.1                |
|                   | \(^{17}\text{O}\) | 3               | 1           | 15.1                |
| Rat F             | \(^{2}\text{H}\)\(^{17}\text{O}\) | 510            | 1           | 15.1                |
| Rat G\*           | \(^{2}\text{H}\)\(^{17}\text{O}\) | 332            | 1           | 15                  |
| Rat H\*           | \(^{2}\text{H}\)\(^{17}\text{O}\) | 367            | 1           | 15                  |

Pop. Mean ± SD: 312 ± 93 g 15 ± 0.6 min

Within 54 min 57 s (see Table 1 for individual inhalation durations), including a ~38 min long postinhalation acquisition (ie, the \(^2\text{H}\)\(^{17}\text{O}\) washout period).

In a subgroup of 2 animals (Table 1) (animals G and H, referred to as low-resolution protocol), the same FOV was scanned with an acquired matrix of 9 × 7 × 7, leading to a voxel size of 77.3 μL by spatial response function adjustment with \(n_{\text{max}} = 45\) averages at the k-space center (a total of 2,048 FIDs or 441 k-space points per CSI volume) and 10.1 s acquisition per 3D CSI volume. Natural abundance \(^2\text{H}\)\(^{17}\text{O}\) CSI volumes \((n = 50)\) were acquired within ~8 mins 24 s, and the same acquisition duration of 54 min 57 s was used to acquire 327 volumes of inhalation data. Other acquisition parameters remained the same.

Postmortem CSI-acquisitions were performed without k-space weighting (12 ms TR and 70° flip angle) and with a pulse length of 400 μs. A FOV of 27.5 × 12.5 × 25 mm\(^3\) was sampled with a matrix of 41 × 19 × 25 voxels (nominal voxel size 0.44 μL). Approximately a total of 2.5 million FIDs, with 1000 points each and a spectral bandwidth of 100 kHz, were acquired in 8 h 18 min.

### 3.4 Brain coregistration and tissue selection

The 3D \(^{17}\text{O}\)-CSI data were coregistered with \(^1\text{H}\) anatomic images and high-resolution (postmortem) \(^2\text{H}\)\(^{17}\text{O}\) images with the same FOV as illustrated in Figure 1A-C. Equally sized regions of interest (ROI) were selected (~3 mm Bregma)\(^{27}\) for brain and in lateral muscle compartments in the same coronal...
slices. The topography of the temporalis muscle was verified anatomically,28-30 and left and right lateral ROIs (42.4 μL, n = 40 voxels after zero-filling in animals A-F; 49.4 μL, n = 28 voxels after zero-filling in animals G-H) were chosen as a subset of the temporalis volume (0.422 mL),31 carefully avoiding partial volume contamination from adjacent brain tissue.

3.5 | Postprocessing, in vivo T₂* estimation and metabolic fitting

Acquired CSI datasets were Fourier-transformed, and the peak of the magnitude spectrum of H₂¹⁷O after apodization (T₂* = 1.8 ms in time domain) was normalized to the natural abundance H₂¹⁷O concentration of 16.3 μmol/g wet tissue of both muscle and brain, assuming equal H₂¹⁷O concentrations (ie, water content) in muscle and brain tissue.18,32-34 Calibration was performed through normalization from the previously defined natural abundance acquisitions of each rat, using the last 20 CSI volumes for the high-resolution and the last 40 volumes for the low-resolution protocol, and pre-inhalation time points (phase 1) were 12 CSI volumes and 23 volumes, respectively. Signals were smoothed by a nearest neighbor moving average (3 adjacent CSI time points).7

Separately, for each rat the data of 2 rats in the low- and high-resolution protocols were phased, and the localized semilogarithmic FIDs were fitted against time for in vivo T₂* relaxation measurement as described in detail in Ref. 22. The metabolic model was fitted according to Equation (1) using a nonlinear least-squares algorithm (Curve Fitting Toolbox 3.5.6, MatLab; MathWorks, Natick, MA, version 9.3.0.713579 (R2017b)) to the H₂¹⁷O signal time courses of tissue signal (inhalation times independent variable; CMRO₂ for brain and RMRO₂ for muscle, KG, KL as dependent variables).

3.6 | Estimation of CBF

CBF was estimated from the same brain ROIs based only on the H₂¹⁷O signal after the end of the inhalation (ie, ~38 min of washout). The previously validated washout model12 is based on the return of local H₂¹⁷O overproduction to a new systemic equilibrium in relation to the rest of the body (VO₂). The concentration of brain tissue H₂¹⁷O using mono-exponential fitting against time courses can be described by the following equation12,35:

\[ Cb(t) = k_3 \times \exp\left(\frac{CBF \times t}{k_1}\right) + k_4. \]
The primary decay constant, proportional to CBF/k₁, can be converted by multiplication with 1.86 to absolute CBF units of mL/g/min (whereas k₃ and k₄ are scale factors). Next, the 2 groups with different ventilation mixtures are compared (N₂ vs. N₂O).

All results are reported in mean ± SD.

4 | RESULTS

Proton structural images showed a clear anatomical contrast between brain and muscle tissue (Figure 1A). Coregistered geometry of ¹⁷O contrast in both in vivo (Figure 1B) and ex vivo ¹⁷O high-resolution images (Figure 1C) matched the anticipated intensity distribution of the ¹⁷O surface coil, that is, stronger ¹⁷O water signal at the surface and in the quadrature B₁ field overlap region in the brain. Figure 2 illustrates representative natural abundance H₂¹⁷O spectra summed over the ROIs before inhalation from low-resolution (Figure 2A) (10 s acquisition averaging) and high-resolution (Figure 2B) (30 s acquisition averaging) ¹⁷O MRSI, indicating a high SNR offered at 16.4 T, in particular, in the brain.

4.1 | Simulation of parameterized H₂¹⁷O dynamics

The simulation results shown in Figure 3A demonstrate the sensitivity of the parameters of the 3-phase model, in particular the K_L or K_G values on the H₂¹⁷O dynamics, which represent the strong influence of perfusion. Time courses of the simulated ¹⁷O signal with varying metabolic rate are shown in Figure 3B for 4 different metabolic rates, exemplifying representative values for the brain and the muscle.

The simulated metabolic rates at different levels showed a qualitatively distinct shape of the H₂¹⁷O signal dynamics at low metabolism (ie, sigmoidal). Despite significant differences in the early phase 2 (phase 2A), the slopes converge in a nonlinear way during the late phase 2 (phase 2B), as shown in Figure 3B. The simulation results indicate that the early dynamic change of the tissue H₂¹⁷O signal after inhalation of ¹⁷O₂ gas is more sensitive to the local metabolic rate than that of late phase 2.

A novel observation from this simulation was that the same K_G/K_L ratio leads to the same equilibrium level of H₂¹⁷O signal at the end of phase 3 (Figure 3A for brain and 3B for muscle at K_G/K_L ratio = 1.5). This suggests that even if the oxygen metabolic rates vary greatly in different tissues (eg, brain vs. muscle), the relative contributions of the H₂¹⁷O signal gain and signal loss due to recirculation and perfusion in different voxels remain the same. Thus, the voxels containing different tissue types will eventually reach the same H₂¹⁷O concentration level.

4.2 | Metabolic rate estimates for brain and resting skeletal muscle tissue

As shown in Figure 4, the H₂¹⁷O signal intensity in muscle ROIs grew in a slower fashion and then accelerated during the late phase 2 (ie. phase 2B) before approaching saturation after the inhalation ended. An absent H₂¹⁷O signal decrease in muscle tissue during the post-inhalation phase due to competing processes between H₂¹⁷O recirculation and washout was in stark contrast to the obvious H₂¹⁷O signal decay observed in the...
brain ROIs (Figure 4B). Reproducible time courses were observed during 3 repeated inhalation measurements in the same animal and MR imaging session (Figure 4C).

Fitting the metabolic rates of brain ROIs, an overall average of $\text{CMRO}_2 = 1.97 \pm 0.19 \, \mu\text{mol/g/min}$ ($n = 26$ ROIs from all 8 rats) was determined. For the 2 subgroups consisting of 4 rats each, a $\text{CMRO}_2$ of $2.07 \pm 0.15$ ($n = 14$ ROIs) and slightly lower $1.84 \pm 0.14 \, \mu\text{mol/g/min}$ ($n = 12$ ROIs) were estimated with $\text{N}_2$ and $\text{N}_2\text{O}$, respectively (Table 2); and no significant differences between the 2 hemispheres were detected. In

**FIGURE 3** (A) Simple iso-metabolic comparison of varying degrees of perfusion and circulatory parameters (ie, the $K_0$ and $K_L$ parameter values) using the 3-phase model (Equation (1)) highlighting phases in high metabolizing tissue: phase 1: preinhalation natural abundance $\text{H}_2\text{H}_2\text{O}^17\text{O}$; phase 2: during inhalation with initial increases dominated by locally produced $\text{H}_2\text{H}_2\text{O}^17\text{O}$ (phase 2A as the first ~2-4 min) and subsequently varied slopes (especially in later phase 2B) from the different perfusion and circulation parameters; and phase 3: postinhalation $\text{H}_2\text{H}_2\text{O}^17\text{O}$ with varying washout rates to reach equilibrium levels linearly affected by the $K_0 / K_L$ ratio at the same local metabolic rate. (B) Zoomed (~2×)-in time 3-phase model plots at different metabolic rates. During the first few min, the $\text{H}_2\text{H}_2\text{O}^17\text{O}$ water content in low metabolizing tissue increases much slower than that in the higher ones (phase 2A); signals approach a similar slope during phase 2B; and at the end of the inhalation, the $\text{H}_2\text{H}_2\text{O}^17\text{O}$ in low metabolizing tissue continues to rise with gradually decreased slope. Both high and low metabolizing tissue approach the same equilibrium due to recirculation of body water at a new steady-state level determined by the global metabolic rate ($\text{VO}_2$, according to Equation (2)). Unless otherwise stated in the legend, all time courses in (B) had $K_0 = 0.3$ and $K_L = 0.2$. The dashed and dotted lines indicate the beginning and end of the phase 2, respectively. $K_0$, $K_{\text{Gain}}$ parameter; $K_L$, $K_{\text{Loss}}$ parameter.
muscle ROIs, an average RMRO₂ of 0.32 ± 0.12 μmol/g/min (n = 23 ROIs) was determined, with some notable intrasubject left and right lateral differences. The estimated muscle oxygen metabolic rates were only one-sixth that of the brain. The perfusion- and diffusion-related parameter Kᵢ was higher than the parameter Kᵢ for both tissue types (for brain: averaged Kᵢ = 0.34 ± 0.05, Kᵢ = 0.22 ± 0.03, n = 26; and for muscle: Kᵢ = 0.63 ± 0.33, Kᵢ = 0.40 ± 0.17, n = 23). Group averages for brain tissue were Kᵢ = 0.34 ± 0.04 (n = 14) for N₂ and Kᵢ = 0.34 ± 0.07 (n = 12) for N₂O. without
In contrast, K_L = 0.20 ± 0.02 (n = 14) for brain within the N_2 group increased by +22% to KL = 0.24 ± 0.02 (n = 12) in the N_2O group, with statistical significance (2-sided unpaired t test at P < .005).

The overall ratio of KG/K_L determined within sessions was 1.51 ± 0.23 (n = 23 ROIs) for muscle and 1.58 ± 0.23 (n = 26 ROIs) for brain tissue, respectively; no statistically significant difference between the 2 tissue types was observed. The same KG/K_L ratios between the brain and muscle converged to the same level of equilibrium H_2^{17}O signal at the later phase 3 (Figure 4B) despite > 6 times of difference in the metabolic rate between the 2 tissues. This finding is in agreement with the prediction from the simulations shown in Figure 3.

### 4.4 In vivo T_2* in muscle and brain tissue

Figure 5 shows the lower T_2* of H_2^{17}O in muscle tissue and ~40% higher T_2* in brain tissue, which are correlated against the independent metabolic rates in the 2 types of tissues. In the same rats, a more than fivefold difference in metabolic rate between muscle and brain is apparent.

### 5 DISCUSSION

This study demonstrates 3 perspectives about the utility of the noninvasive and quantitative ^17^O MRSI or MRI method with inhalation of ^17^O_2 gas, determining the oxygen consumption rates in organs with higher and/or lower metabolic activity, measuring the systemic global oxygen consumption

| Individual Animal | RMRO_2 Left Muscle ROI | RMRO_2 Right Muscle ROI | CMRO_2 Left S1 ROI | CMRO_2 Right S1 ROI |
|-------------------|------------------------|------------------------|--------------------|--------------------|
| Rat A             | 0.15                   | 0.11                   | 2.11               | 2.08               |
| Rat B             | 0.28                   | 0.43                   | 2.03               | 1.90               |
| Rat C             | 0.27                   | 0.30                   | 2.02               | 2.08               |
|                   | 0.33                   | 0.31                   | 2.06               | 2.01               |
| Rat D             | 0.32                   | *                      | 2.30               | 2.45               |
|                   | 0.37                   | *                      | 1.85               | 1.96               |
| Mean ± SD         | 0.30 ± 0.07            | 0.27 ± 0.12            | 2.07 ± 0.14        | 2.07 ± 0.18        |
| Rat E             | 0.45                   | 0.34                   | 1.82               | 1.77               |
|                   | 0.45                   | 0.16                   | 1.82               | 1.83               |
|                   | 0.46                   | 0.42                   | 1.68               | 1.74               |
| Rat F             | 0.13                   | *                      | 2.14               | 2.08               |
| Rat G†            | 0.44                   | 0.57                   | 1.86               | 1.89               |
| Rat H†            | 0.34                   | 0.20                   | 1.80               | 1.69               |
| Mean ± SD         | 0.38 ± 0.11            | 0.34 ± 0.14            | 1.85 ± 0.15        | 1.83 ± 0.14        |
| Mean values of right and left ROIs | RMRO_2 = 0.32 ± 0.12‡ | CMRO_2 = 1.97 ± 0.19‡ |

CMRO_2: cerebral metabolic rate of oxygen consumption; RMRO_2, resting metabolic rate of oxygen consumption in skeletal muscle; ROI, region of interest.

*No convergence of the fitting procedure.
†This subgroup of 2 animals was acquired at a higher temporal resolution (10 s per 3D CSI volume) with the lower spatial-resolution protocol.
‡P < .01 significant tissue-type difference between muscle and brain (paired t test).
rate using a steady-state model, and characterizing local CBF under 2 experimental conditions.

### 5.1 Modeling dynamics of H$_2^{17}$O signal in muscle and brain

We have simulated the H$_2^{17}$O signal dynamics using an animal-adapted 3-phase metabolic model as described by Equation (1) using different parameter settings to mimic the experimentally measured H$_2^{17}$O time courses (simulation in Figure 3). The simulation data indicate that the initial change of the tissue H$_2^{17}$O signal during the early inhalation period (phase 2A) is dominated by the metabolically produced H$_2^{17}$O, and the contribution from recirculating H$_2^{17}$O is small. Therefore, the initial slope of the H$_2^{17}$O concentration in phase 2A is sensitive to the oxygen metabolic rate of the tissue,36 which is much slower in muscle as compared to the steeper increase in brain tissue. Despite the expected differences in the local metabolic rate, the time course of the H$_2^{17}$O signal in the late inhalation phase (phase 2B) converged to a relatively similar slope for all tissues (Figures 3, 4) (Supporting Information Figure S1B). The contribution of recirculating water increased with inhalation time and gradually dominated the H$_2^{17}$O signal in the later phase of the inhalation, resulting in converging slopes between high- and low-activity tissues as observed in experimental data.

![FIGURE 5](image)

**FIGURE 5** Scatter plot of in vivo tissue T$_2^*$ versus metabolic rate showing high correlation in both tissue types of brain and muscle from clearly separated clusters of the independent properties of metabolic rate and relaxometric behavior inside ROIs. The distinction benefits from the fact of a stark difference in T$_2^*$, as also previously reported for both in vivo and postmortem tissues. Each cluster is based on the pooled tissue type of 2 representative rats (rat A and rat G), of high-resolution and low-resolution protocols, respectively. Note: this is only an observation to confirm the accurate selection and size (ie, partial volume contamination) of the ROIs and does not imply a causal relation between relaxation rate and metabolic rate in either direction.

### TABLE 3

Summary of VO$_2$,average (μmol/g tissue/min) and cerebral blood flow (mL/g tissue/min) results based on washout in brain

| Individual Animal | VO$_2$,average Left and Right Muscle ROI | VO$_2$,average Left and Right Brain ROI | CBF Left Brain ROI | CBF Right Brain ROI |
|-------------------|-----------------------------------------|----------------------------------------|-------------------|---------------------|
| Rat A             | 1.17                                    | 1.19                                   | 0.27              | 0.27                |
| Rat B             | 1.21                                    | 1.04                                   | 0.28              | 0.25                |
| Rat C             | 1.19                                    | 1.16                                   | 0.26              | 0.28                |
| Rat D             | 1.39                                    | 1.09                                   | 0.31              | 0.28                |
| Mean ± SD         | 1.19 ± 0.10**                           | 1.17 ± 0.10**                          | 0.28 ± 0.02**     | 0.27 ± 0.02**       |
| Rat E             | 1.14                                    | 1.11                                   | 0.41              | 0.33                |
| Rat F             | 0.64                                    | 0.91                                   | 0.32              | 0.33                |
| Rat G*            | 1.17                                    | 1.13                                   | 0.32              | 0.32                |
| Rat H*            | 0.74                                    | 0.73                                   | 0.46              | 0.38                |
| Mean ± SD         | 0.96 ± 0.22**                           | 0.98 ± 0.15**                          | 0.36 ± 0.06**     | 0.32 ± 0.05**       |
| Overall average of both N$_2$ and N$_2$O | VO$_2$,average (n = 13) 1.08 ± 0.20 μmol/g body/min | VO$_2$,average (n = 13) 1.08 ± 0.16 μmol/g body/min | CBF (n = 26) 0.30 ± 0.05 mL/g tissue/min |

CBF, cerebral blood flow; VO$_2$, basal body metabolic rate of oxygen.

*This subgroup of 2 animals was acquired at a higher temporal resolution (10 s per 3D CSI volume) and with lower spatial resolution protocol.

**P < .05 significant population difference between N$_2$ (rats A-D) and N$_2$O (rats E-H) groups (unpaired t test).**
5.2 Determining the oxygen metabolic rates in muscle and brain

Despite the limited spatial specificity, arteriovenous difference measurements can still be regarded as the gold standard for oxygen consumption measurements. However, due to their invasiveness, they are less convenient, and the variability of draining vascular territory effects on reproducibility motivates the use of noninvasive alternatives such as $^{17}$O MRSI/MRI with $^{17}$O tracer inhalation, as in parallel has been attempted through $^{15}$O PET. By fitting the $H_2^{17}$O signal dynamics of the rat muscle ROIs to the adapted 3-phase metabolic model, the resting-state metabolic rate of oxygen consumption in skeletal muscle ($RMRO_2$) was $0.32 \pm 0.12 \mu mol/g/min$. Comparing to the literature reports of oxygen metabolic rates in skeletal muscle from the earliest in vitro estimates to more recent studies in Wistar rats, the results of the present study show a good agreement with the literature values (Table 4). Perfused rat hindquarter muscle metabolic rate was reported similar (e.g., $0.37 \mu mol O_2/g/min$) depending on modality. Other differences could be inherent to the heterogeneity of muscle fibers, which in the case of the temporalis muscle is low compared to other muscles (e.g., soleus or gastrocnemius) and in other species. To the best of our knowledge, this is the first to report measurements of oxygen metabolic rates using $^{17}$O MR imaging for resting skeletal muscle, although working cardiac muscle with a high oxygen consumption rate has been shown before in isolated heart as well as in vivo rat heart. In muscle, alternative pathways (i.e., fatty acids) are possible in contrast to the glucose-based metabolism of the brain. However, both are based on oxygen as the substrate in the predominant mitochondrial electron transfer chain as origin of metabolic $H_2^{17}$O. Therefore, this study is in agreement with previous measurements in the cardiac muscle, both perfused and in vivo, but extends to a much lower regime of metabolic rates in the immobilized, resting skeletal muscle, with very distinguishable characteristics.

The averaged CMRO$_2$ value $(= 1.97 \pm 0.19 \mu mol/g/min)$ as determined in this study is in agreement with the value $(= 2.19 \pm 0.14 \mu mol/g/min)$ from a literature report in the rat brain under relatively lower dose $\alpha$-chloralose anesthesia obtained with a different modeling and experimental protocol. These comparisons provide strong evidence to support the validity and reliability of the quantitative $^{17}$O MRS imaging method, as described in this work for noninvasively imaging oxygen metabolic rates in the brain and resting muscle with a very low metabolic activity. Thus, we conclude that the same imaging approach should be applicable for most organs across a wide range of metabolic rates.

5.3 Global systemic metabolic rates

It should be reasonable to assume that the metabolite pools are in equilibrium upon a stable physiological condition of the animal. As observed in both simulation and experimental data, the post-inhalation $H_2^{17}$O concentrations of different ROIs containing brain or muscle tissue eventually converged to the same steady-state level, which represented the new equilibrium $H_2^{17}$O concentration after the $^{17}$O$_2$ inhalation. Based on that information and Equation (2), we were able to derive the global systemic metabolic rate. Metabolic inter- or intersubject fluctuations are likely caused by variations in the physiological animal condition (i.e., ventilation parameters, anesthesia status, and body weight). Thus, in contrast to other studies, our estimates of the average global oxygen metabolic rate (Table 3) were

| Species/Technique   | Muscle MRO$_2$ $\mu mol/g/min$ | Skeletal Muscle Type | Literature Reference |
|---------------------|---------------------------------|----------------------|----------------------|
| Rat/perfused        | 0.65                            | Global muscle estimate | Field et al. 1939\cite{39} |
| Rat/perfused        | 0.37                            | Hindquarter          | Hood et al. 1986\cite{41} |
| Rat/perfused        | 0.23                            | Hindquarter          | Rolfe & Brand 1996\cite{40} |
| Rat/perfused        | 0.75                            | Spinotrapezius       | Behnke et al. 2002\cite{42} |
| Rat/this study      | 0.32                            | Temporalis muscle    | –                     |
| Human/invasive $\Delta A/V$ | 0.13                        | Whole leg            | Oikonen et al. 1998\cite{60} |
| Human/$^{15}$O$_2$ inhalation PET | 0.11                        | Whole leg            | Oikonen et al. 1998\cite{60} |
| Human/$^{15}$O$_2$ inhalation PET | 0.10                        | Whole leg            | Nuutila et al. 2000\cite{64} |
| Human/$^{15}$O$_2$ inhalation PET | 0.05                        | Whole leg            | Ilkka et al. 2011\cite{57} |

Comparison between selected muscle metabolism estimates using different techniques in rodents and humans, with the latter being more similar to the $^{17}$O$_2$ inhalation technique used in this study.

MRO$_2$, metabolic rate of oxygen.
robust and consistent, independent of whether they were inferred from brain or muscle ROI time courses. Previous studies have used $^{17}$O to assess the total metabolic rate of oxygen thus far in dogs$^{50}$ and mealworms.$^{51,52}$ Very early studies on Wistar rats$^{39}$ measured oxygen consumption in muscle in vitro, with more recent reports estimating VO$_2$ for muscle of 18.7 mL O$_2$/kg/min in anesthetized rats$^{53}$ and 24.5 ± 8.5 mL O$_2$/kg/min in awake rats of the same strain, remarkably close to our results (24.2 mL O$_2$/kg/min).$^{54}$ The variations in literature values also highlight possible inter-subject variations and different approaches used for these studies.$^{55-57}$

5.4 Increased washout of locally produced H$_2^{17}$O during N$_2$O ventilation

The washout of H$_2^{17}$O in brain tissue during the post-inhalation period (ie, related to perfusion or CBF) has been established previously.$^{12}$ It reflects the dynamics of perfusion washout of the metabolically produced H$_2^{17}$O in brain tissue and an inflow of global recirculating H$_2^{17}$O. However, there is no observable “washout” in the lower metabolizing muscle tissue below the average body oxygen metabolic rate (VO$_{2\text{average}}$ ~1.1 μmol/g/min), presumably due to a substantial inflow effect from recirculating H$_2^{17}$O and low metabolic activity. Thus, in contrast to brain tissue, a significant extent of “wash-in” from systemic recirculation after the $^{17}$O$_2$ inhalation was observed in muscle (Figure 4, from t = 15 min onwards).

An increase in CBF through vasodilation has been observed and reported before with high percentage N$_2$O administration.$^{58}$ Thus, the anesthetic properties and vasodilatory effects of N$_2$O may reduce the global metabolism and possibly uncouple it partially from the narrowly regulated cerebral local oxygen metabolism.$^{59}$

5.5 Validation of the 3-phase model in future research

Although the influence of recirculating metabolic water is substantial depending on the regional and global organism rates, the 3-phase model accounts accurately for the metabolic rate differences between tissue types. Our measurements used long inhalation times of over 15 min, thus, requiring a nonlinear metabolic model.$^2$ It can also be concluded that the longer duration of the inhalation phase does not linearly increase the CMRO$_2$ measurement sensitivity; it is limited by the accumulation of recirculating total body H$_2^{17}$O.

An internal ROI validation confirmed whether the voxels that were selected truly reflected the chosen tissue type by assessment of T$_2^*$ against metabolic rate in brain and muscle. Figure 5 shows a plot of the independent properties of tissue T$_2^*$ and metabolic rate values for the ROIs taken from muscle and brain under the 2 different $^{17}$O MRS imaging protocols (low- vs. high-resolution protocol). Two well-separated clusters associated with the 2 types of tissues due to stark difference in transverse relaxation between the tissues (a much longer T$_2^*$ in brain than that of muscle)$^{22}$ confirm the ROI placements. Particularly, the muscle ROIs covered the temporalis muscle sufficiently accurately. It also demonstrates that the strong divergence in metabolic rate reflects an underlying tissue difference. However, this approximate separation is only possible due to the significantly shorter T$_2^*$ values of H$_2^{17}$O in muscle than in brain tissue.$^{22}$

It also should be noted that certain metabolic rate variability stems from tissue heterogeneity within ROIs. For example, in brain tissue estimates, despite low intrasession variance (eg, see rat A) a hemispheric difference was likely induced by ROI placement near the boundary between brain and muscle tissues, leading to partial volume effects. Another technical limitation is the relatively low SNR of $^{17}$O signal detected in the muscle due to short T$_2^*$ and lateral differences in B$_1$, resulting in ~half SNR than that of brain tissue (see the $^{17}$O spectra in Figure 2A,2B). Therefore, the fidelity in imaging muscle could be improved, for instance, using a coil array covering both brain and muscle with optimal detection sensitivity.

Finally, we would anticipate smaller variations of the $^{17}$O MRSI approach when potentially activating the muscle by stimulation, as was done in a different paradigm during varying workload for instance, in cardiac muscle,$^{8,10}$ resulting in an elevated oxygen metabolic rate. In previous brain experiments, with an implantable $^{17}$O RF coil, the measurement of an arterial input function and the measurement of blood flow through H$_2^{17}$O bolus measurements was used for a detailed investigation, which also allowed the calculation of oxygen extraction fraction.$^3$ Thus, in future studies in other rat muscles (eg, in the leg, by implantation of an arterial $^{17}$O RF coil on the femoral artery or separately on the tail artery), the metabolic rate could be validated after electrical stimulation over a wide range of metabolic rates and perfusion. Dynamically measuring the increased metabolic rate during $^{17}$O$_2$ inhalations could give new insights to different muscle fiber types. Furthermore, we would expect a simultaneous measurement to be robust in consideration of systemic changes in animal physiology.

6 Conclusion

In this study, we have extended the applicability of in vivo $^{17}$O MR imaging to measure and image the resting skeletal muscle with a very low oxygen metabolic rate (~16% of the brain tissue). We have also confirmed the consistency of the CMRO$_2$ results measured during prolonged and repeated inhalations of $^{17}$O$_2$ gas in this study with previous findings.
Because the brain has a very high metabolic rate of oxygen consumption, in contrast to the very low rate in the resting muscle, we anticipate that the same $^{17}$O MR imaging approach and modeling will be useful for other organs such as liver and heart. Therefore, we expect a broad impact of using the $^{17}$O MR imaging technology for metabolic rate measurements in normal and diseased organs beyond the brain.

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**SUPPORTING INFORMATION**

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

**FIGURE S1** A, B: Zoomed views of the signal time courses around the beginning (A) and end (B) of the inhalation, for
different values also show the different time points as with the different resolution protocols (10 s vs. 30 s per CSI volume, with the latter in close resemblance to the experimental design in Ref. 4 [42 s per 3D CSI volume]). The low $p_{\text{human}}$ causes a substantially delayed reaction at both beginning and end of the inhalation, with less effect at an increase of a hypothetical 7 min$^{-1}$.