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Estimation of the regional cerebral metabolic rate of oxygen consumption with proton detected ^{17}O MRI during precision $^{17}\text{O}_2$ inhalation in swine

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Abstract

Despite the importance of metabolic disturbances in many diseases, there are currently no clinically used methods for the detection of oxidative metabolism *in vivo*. To address this deficiency, ^{17}O MRI techniques are scaled from small animals to swine as a large animal model of human inhalation and circulation. The hemispheric cerebral metabolic rate of oxygen consumption (CMRO_2) is estimated in swine by detection of metabolically produced H_2^{17}O by rapid $\text{T}_{1\rho}$ -weighted proton magnetic resonance imaging on a 1.5 Tesla clinical scanner. The ^{17}O is delivered as oxygen gas by a custom, minimal-loss, precision-delivery breathing circuit and converted to H_2^{17}O by oxidative metabolism. A model for gas arterial input is presented for the deeply breathing large animal. The arterial input function for recirculation of metabolic water is measured by arterial blood sampling and high field ^{17}O spectroscopy. It is found that minimal metabolic water “wash-in” occurs before 60 seconds. A high temporal resolution pulse sequence is employed to measure CMRO_2 during those 60 seconds after delivery begins. Only about one tidal volume of ^{17}O enriched oxygen gas is used per measurement. Proton measurements of signal change due to metabolically produced water are correlated with ^{17}O *in vivo* spectroscopy. Using these techniques, the hemispheric CMRO_2 in swine is estimated to be $1.23 \pm 0.26 \mu\text{mol/g/min}$, consistent with existing literature values. All of the technology used to perform these CMRO_2 estimates can easily be adapted to clinical MR scanners, and it is hoped that this work will lead to future studies of human disease.

Keywords

Oxygen-17; ^{17}O MRI; ^{17}O MRS; Cerebral Metabolic Oxygen Consumption; Arterial Input Function

Introduction

Alterations in oxygen metabolism are known to occur in many diseases; however limitations of existing technologies prevent clinical studies based on this potentially important diagnostic information. For example, Positron Emission Tomography (PET) with ^{15}O requires an onsite

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cyclotron not available at most clinical sites to generate short half-life radioactive tracers. Furthermore, several experiments with multiple tracers and sophisticated modeling is necessary to generate metabolic information in disease states (Ibaraki et al., 2004; Mintun et al., 1984). Quantitative functional MRI techniques rely on numerous assumptions about the coupling of blood flow, volume, and oxidative metabolism that become highly difficult to predict during pathologic states (Davis et al., 1998). Measurements with functional MRI are only relative to some change after a baseline measurement and do not give the absolute cerebral metabolic rate of oxygen consumption (CMRO₂). With this motivation, numerous groups have attempted to use the only naturally occurring, NMR active, non-radioactive isotope of oxygen, ¹⁷O, to obtain *in vivo* information about metabolism by inhalation of ¹⁷O₂.

Inhaled ¹⁷O₂ gas is invisible to MRI. This is assumed to be due to its extremely fast relaxation. The H₂¹⁷O produced by metabolism is then detected by two distinct MR techniques termed direct and indirect. The direct techniques for H₂¹⁷O detection utilize MR spectroscopy or imaging at the Larmor frequency of ¹⁷O. *In vivo* studies using the direct techniques have shown the possibility of detecting ¹⁷O *in vivo* (Mateescu et al., 1987) and have measured metabolism (Arai et al., 1990; Fiat et al., 1992), flow (Arai et al., 1998; Kwong et al., 1991), or both (Arai et al., 1991; Pekar et al., 1991). Two groups have been able to demonstrate decreased ¹⁷O metabolism in the setting of hypothermia, one in insects (Mateescu and Cabrera, 1997) and more recently using 3D spectroscopy on rats at 9.4T (Zhu et al., 2007). Still, direct ¹⁷O is difficult to perform due to the relative unavailability of high field broadband scanners, low gyromagnetic ratio of ¹⁷O, and the fast relaxation and low natural abundance of H₂¹⁷O (Zhu et al., 2005).

Alternatively, indirect imaging relies on the strong spin-spin coupling of ¹H and ¹⁷O in water to shorten the T₂ of ¹H in bulk water (Meiboom, 1961). This relaxation enhancement can then be used to calculate the local H₂¹⁷O concentration (Stolpen et al., 1997). In addition, there are several methods for decoupling the ¹H-¹⁷O interactions to provide control images during scanning. One method uses irradiation at the ¹⁷O frequency to achieve decoupling (Ronen et al., 1997). This was utilized to image metabolism (Ronen et al., 1998) and flow (de Crespigny et al., 2000) in rats. These techniques require double-tuned hardware and a second transmit channel often found on small animal scanners but rarely found with clinical scanners. High radiofrequency power required on the ¹⁷O decoupling channel makes this difficult to implement without violating tissue power absorption limits. Another method of decoupling is proton frequency irradiation by spin locking (T_{1ρ}) either on (Reddy et al., 1995) or off (Charagundla et al., 1998; Sood, 2004) resonance, where high amplitude spin locking decouples ¹H from ¹⁷O and low amplitudes provide T₂ contrast. These techniques have been used to measure flow (Tailor et al., 2003) and metabolism (Tailor et al., 2004) in rats.

Still, since the vast majority of ¹⁷O studies have been performed on small animals, the objective of this study is the measurement of CMRO₂ with clinical hardware with the goal of human studies using ¹⁷O₂. As such, adolescent swine that are the size of an adolescent human are used, with similar cardiovascular and respiratory systems to an adult human, in a 1.5 Tesla clinical scanner. The scaling of metabolic measurement by ¹⁷O to large animals and humans presents some unique challenges. First, ¹⁷O₂ gas is expensive, and the body sizes of large animals dictate much more tracer must be used than in small animals. Therefore, short time course inhalation is used here for large animals, the shortest yet reported for ¹⁷O₂, and the uptake is modeled for slowly breathing, large lung volume, large circulation volume animals. A low loss, precision breathing circuit is employed for this delivery. With such a short time course of inhalation, fast sequences must be used with sufficient contrast-to-noise, signal-to-noise, and spatial resolution to make measurements. As such, a low amplitude T_{1ρ} single-shot readout sequence is used (Mellon et al., 2008). This must be used without high amplitude T_{1ρ} decoupling due to radiofrequency power amplifier limitations on our clinical scanner that

are common to most clinical scanners. Still, decoupling can be used with these same techniques if a high spin lock amplitude or second transmit channel is achievable, but with magnet stability and without subject motion decoupling may not be necessary as control images may be taken immediately before the delivery of $^{17}\text{O}_2$ gas.

Similar scaling issues hinder the human application of direct ^{17}O techniques at high field as well. For example, while ^{17}O in H_2^{17}O has a short T_1 which allows for very rapid acquisition and averaging in small animals, specific absorption rate limits applied to humans partially hinders this advantage. It is well established that the low sensitivity of the nucleus presents SNR limitations for detection. Unfortunately, the dramatic signal to noise gain granted by using very small radiofrequency coils on small animal heads is abolished unless very superficial H_2^{17}O measurements are made in humans with small coils. Further, at least as much ^{17}O gas, scaled for body weight, will be required and the efficiency and precision of delivery should be considered in that case as well.

One additional consideration is required. To arrive at the CMRO_2 , the arterial input function must be known for the recirculation of metabolic H_2^{17}O (mH_2^{17}O) produced elsewhere in the body. This recirculation must be either modeled (Tailor et al., 2004; Zhang et al., 2004), measured (Zhang et al., 2003), or otherwise accounted for in order to obtain CMRO_2 . In large animals there will be a time delay between the start of mH_2^{17}O generation in parenchyma and the start of incoming metabolically generated water washed in from other tissues after $^{17}\text{O}_2$ delivery. During this time delay, PET studies have estimated CMRO_2 in normal subjects (Meyer et al., 1987). While an analogous recirculation delay for ^{17}O is expected and has been suggested (Pekar et al., 1991), no group has combined ^{17}O delivery in a large animal model with fast imaging techniques to further investigate the possibility of measuring CMRO_2 from the pre-recirculation time.

Therefore, the three purposes of this work are: (i) to demonstrate the combined use of low loss precision breathing delivery circuit to minimize ^{17}O gas use combined with fast imaging on clinical scanners to measure the kinetics of metabolically produced H_2^{17}O in large animals. (ii) Determine the time delay before significant wash-in of H_2^{17}O occurs from other regions. (iii) Show that CMRO_2 can be estimated in large animals by measuring the formation of mH_2^{17}O in brain during the recirculation delay with single-shot low amplitude $T_{1\rho}$ imaging. In total, this work provides a basis for future ^{17}O studies in humans.

Theory

Modeling of inhaled atomic percentage of $^{17}\text{O}_2$ during the first minute

It is necessary to know or model the atomic percentage of ^{17}O in the oxygen metabolized by cells because while the mH_2^{17}O rate of formation is measureable by MRI, the fractional enrichment of the $^{17}\text{O}_2$ generating that water at the cellular level during that time is not known. This is only a minor concern for small animals that breathe very rapidly and therefore equilibrate their lung gas with the inhaled gas rapidly. Instead, large animals breathe relatively deeply and slowly. To investigate the expected change in tissue ^{17}O enrichment in a large animal, we generated a simple step-wise model for estimating the fractional enrichment, $f_a(i)$, of ^{17}O in the lungs for each breath. Each step (breath) is modeled as an instantaneous inhalation, mixing of $^{17}\text{O}_2$ in the lungs, and exhalation via a simple dilution model with the addition of O_2 uptake into the body from the lungs.

Let us begin with an inspired volume of ^{17}O , $V_i^{17}\text{O}$. That volume is given by the tidal volume (TV), the fraction of oxygen in that gas ($f_i\text{O}_2$), and the ^{17}O enrichment of that gas (α).

$$V_i^{17}\text{O} = \text{TV} \cdot f_i\text{O}_2 \cdot \alpha \quad \text{Equation 1}$$

The inhaled volume adds to the ^{17}O left in the lungs at the end of the previous exhalation, $V_e^{17}\text{O}$. That lung volume of ^{17}O at completed exhalation is determined by the functional residual capacity (FRC), the fraction of oxygen in that exhaled gas ($f_e\text{O}_2$), and has the ^{17}O enrichment of the breath before it, given by $f_a(i-1)$.

$$V_e^{17}\text{O} = \text{FRC} \cdot f_e\text{O}_2 \cdot f_a(i-1) \quad \text{Equation 2}$$

To calculate $f_e\text{O}_2$ one must approximate the amount of oxygen consumed during the breath. For simplicity it is assumed that the inhalation, mixing, and exhalation happen instantly to produce a step change and the oxygen consumption occurs on the post-exhaled gas. This consumption is given on a per breath basis by,

$$f_c\text{O}_2 = \frac{\text{VO}_2}{\text{RR}} \frac{1}{\text{FRC}} \quad \text{Equation 3}$$

where $f_c\text{O}_2$ is the fraction of the O_2 consumed from the exhaled lung volume during each breath, VO_2 is the per minute volume of O_2 consumed, and RR is the respiratory rate in breaths/min. Hence, to take into account the O_2 consumption in the “exhaled” gas, $f_e\text{O}_2$ is set as in Equation 4.

$$f_e\text{O}_2 = f_i\text{O}_2 - \frac{\text{VO}_2}{\text{RR}} \frac{1}{\text{FRC}} \quad \text{Equation 4}$$

The incoming tidal volume of O_2 mixes with the lung's end exhalation O_2 volume, to create the $f_m\text{O}_2$, or the mixed fraction of O_2 .

$$f_m\text{O}_2 = \frac{(\text{TV} \cdot f_i\text{O}_2) + (\text{FRC} \cdot f_e\text{O}_2)}{\text{FRC} + \text{TV}} \quad \text{Equation 5}$$

The combination of all these factors leads to Equation 6, where $f_a(i)$ is the estimated alveolar ^{17}O enrichment at that breath.

$$f_a(i) = \frac{\overbrace{\text{TV} \cdot f_i\text{O}_2 \cdot \alpha}^A + \overbrace{\text{FRC} \cdot f_e\text{O}_2 \cdot f_a(i-1)}^B}{\underbrace{(\text{TV} + \text{FRC}) f_m\text{O}_2}_C} \quad \text{Equation 6}$$

The numerator in the equation specifies the volume of ^{17}O while the denominator specifies the overall volume of oxygen. To convert $f_m\text{O}_2$ to the overall volume of O_2 in the denominator, it must be multiplied by the total lung volume $\text{TV} + \text{FRC}$. Thus, the part of the equation labeled **A** represents an incoming breath (Equation 1). The part labeled **B** accounts for the uptake of

oxygen into the body from the lungs via Equation 2 and using Equation 4 to take metabolic consumption into account. The part labeled C normalizes the equation to units of fractional enrichment.

Using this model, the first minute of ^{17}O gas enrichment during enriched oxygen inhalation was simulated according to the parameters shown in Table 1. The starred parameters were approximated from literature values. To convert to brain tissue ^{17}O gas enrichment, this simulation estimates that there is a 10 second delay from the inhalation start time until the inhaled oxygen reaches the brain. This is considered a reasonable value because there are two delays in inhaled gas reaching the brain. First, there is a 2.4 second delay from the pump to the airway (Baumgardner et al., 2008). From there, the lung to brain transit time is estimated at 7 seconds (Leggett and Williams, 1995). This is very close to 10 seconds. The results of this simulation, the fractional enrichment of the ^{17}O in the brain tissue after each breath, are shown in Figure 1.

From the average of points at 10 seconds through 59 seconds, these simulations estimate a mean of 58.81% of the delivered gas concentration being metabolized during the CMRO_2 calculation time (10–59sec). This is a significant correction that must be considered when computing CMRO_2 in the next section.

Determination of CMRO_2 after $^{17}\text{O}_2$ inhalation

The theory for calculation of CMRO_2 based on $^{17}\text{O}_2$ is derived from flow measurements originally proposed by Kety & Schmidt (Kety and Schmidt, 1948) and later applied to ^{17}O (Fiat and Kang, 1992; Zhang et al., 2004). As a brief summary, CMRO_2 ($\mu\text{mol/g}$ brain tissue/min) may be solved from,

$$\frac{d}{dt}C_b(t) = \frac{2\alpha(t)}{f_1}(\text{CMRO}_2) + Q[C_a(t) - C_v(t)] \quad \text{Equation 7}$$

where $C_b(t)$ is the mH_2^{17}O concentration of brain water in $\mu\text{mol/g}$ water, f_1 is the weight fraction of water in tissue (g water/g tissue), $\alpha(t)$ is the fractional enrichment of metabolized $^{17}\text{O}_2$, Q is the blood flow rate of that area of tissue, and $C_a(t)$ and $C_v(t)$ are the arterial and venous mH_2^{17}O concentrations respectively. There is a natural abundance of H_2^{17}O in naturally occurring water ($20.35\mu\text{mol/g}$), but it is assumed that is constant throughout the time of the experiment and all change is due to metabolic production. The part of the equation before the addition represents locally produced mH_2^{17}O while the latter part represents outflow and inflow of mH_2^{17}O . In the short time course of $^{17}\text{O}_2$ measurement where there is not significant recirculation of mH_2^{17}O , $C_a(t) - C_v(t)$ approximates 0 during the measurement time. A time of at least one minute is verified in this manuscript and other groups (Meyer et al., 1987; Mintun et al., 1984; Ohta et al., 1992; Pekar et al., 1991) have found similar results (reviewed in the discussion). Then integration of Equation 7 is stated as Equation 8 with the simplification due to insignificant recirculation during the first minute.

$$\Delta C_b = \int_{t_0}^t \frac{2\alpha(t)}{f_1}(\text{CMRO}_2) \quad \text{Equation 8}$$

In the equations ΔC_b is the tissue change in mH_2^{17}O concentration over the time of t_0 to t , the time of the CMRO_2 calculation. As we described in the previous section, $\alpha(t)$ cannot simply

be approximated by α , and instead will be replaced by the average ^{17}O enrichment of the oxygen gas over the time of CMRO_2 calculation, f . Equation 8 is then reduced as follows.

$$\text{CMRO}_2 = \frac{\Delta C_b \cdot f_1}{2f} \quad \text{Equation 9}$$

The framework for *in vivo* detection of ΔC_b is primarily based on the work of Stolpen, Reddy, and Leigh (Stolpen et al., 1997). They begin by stating that observed T_2 for a fluid or tissue is dependent on the T_2 in the absence of ^{17}O , T_2^0 , and the atomic fraction of ^{17}O , C , in the water as follows,

$$\frac{1}{T_2} = \frac{1}{T_2^0} + R_2 \cdot C \quad \text{Equation 10}$$

where $R_2 (=1/T_2)$ is the transverse relaxivity due to ^{17}O in a tissue, for a tissue with a given exchange time. For a 5% gelatin (tissue simulation) phantom, that group measured $R_2=3.28$ per at. % per second with almost no change in R_1 (.01 per at. % per second). This study assumes the relaxivity in tissue brain water is equal to the relaxivity measurements from those gelatin phantoms. From theoretical calculations, those measurements correspond to an exchange time of $\tau=.5\text{ms}$ (Meiboom, 1961). The maximum theoretical R_2 is 18% higher based on an exchange lifetime of 0.9 ms (Stolpen et al., 1997), and that provides some evidence that this estimation places R_2 within 20% of the correct value. Without a more precise measurement of R_2 for individual tissue types this suggests that all single absolute measurements are imprecise to about 20%. Measurements of change between subjects or within subjects would also have 20% uncertainty, assuming that R_2 does not change appreciably between or within subjects. Stolpen et al. also showed that for physiologically relevant concentrations of ^{17}O , R_2 remains a linear factor.

Reddy, Stolpen, and Leigh found in a separate manuscript that $R_2 \approx R_{1\rho}$ at 100Hz (Reddy et al., 1995). That finding provides a basis for replacing TE (echo time in a T_2 sequence) and T_2 with TSL (spin-lock time in a $T_{1\rho}$ sequence) and $T_{1\rho}$. Stolpen, et al derived the equations for the calculation of C during an experiment. For measurements made here the $R_{1\rho}$ was converted to units of $5.96 \times 10^{-6} (\mu\text{mol H}_2^{17}\text{O/g tissue})^{-1} \times \text{ms}^{-1}$ and C takes on the units of $\mu\text{mol H}_2^{17}\text{O/g tissue}$. To follow the work of Stolpen, et al to determine H_2^{17}O oncentration in the absence of decoupling and with low amplitude spin locking, we begin with the signal obtained after $T_{1\rho}$ relaxation of tissue, where S is the signal in the absence of relaxation and S_R is the signal with relaxation. Thus, the observed signal after relaxation with ^{17}O relaxation enhancement is given in Equation 11.

$$S_R = S \cdot e^{\left[-\text{TSL} \left(\frac{1}{T_{1\rho}^0} + R_{1\rho} \cdot C \right) \right]} \quad \text{Equation 11}$$

Instead of using $T_{1\rho}^0$, the relaxation in the absence of ^{17}O , as performed in previous derivations we set a variable $T_{1\rho}^i$, the $T_{1\rho}$ at the beginning of an experiment including the existing (natural abundance) amount of ^{17}O . The amount of ^{17}O in water added during an experiment is termed ΔC . This yields a pair of equations for the signal before the addition of ^{17}O (S_0) and after the addition of ^{17}O (S_t) as follows.

$$S_o = S \cdot e^{\left[\frac{-TSL}{T_{lp}^i} \right]}$$
Equation 12

$$S_t = S \cdot e^{\left[-TSL \left(\frac{1}{T_{lp}^i} + R_{lp} \cdot \Delta C \right) \right]}$$
Equation 13

To solve for the concentration of H₂¹⁷O during an experiment based on the signal before and during ¹⁷O metabolism, we take the ratio of S_t/S_o and rearrange for the concentration change ΔC to produce Equation 14.

$$\Delta C = - \frac{\ln(S_t/S_o)}{TSL \cdot R_{lp}}$$
Equation 14

Combining the unit conversion and tissue gas concentration corrections of Equation 9 with the observed signal changes of Equation 14 yields the final equation used to calculate CMRO₂ for indirect imaging in Equation 15.

$$CMRO_2 = - \frac{\ln(S_t(t)/S_o) f_1}{TSL \cdot R_{lp} \cdot 2f}$$
Equation 15

Until now we described how to solve for a change in H₂¹⁷O water concentration without units of time. For the purposes of measurement during an experiment, the term S_o represents the initial signal while the S_t(t) represents the signal change due to H₂¹⁷O per unit time. In this case signal change is measured over about 50 seconds, but the final measurement is based on the linear fit of change over that time, then extended to one minute to produce signal change per minute.

For direct ¹⁷O spectroscopy, the change in signal is directly proportional to the increase in concentration of mH₂¹⁷O, leading to,

$$CMRO_2 = C_b(0) \frac{(S_t(t)/S_o) f_1}{2f}$$
Equation 16

where C_b(0) is the concentration of H₂¹⁷O at the start of the experiment (in units of μmol H₂¹⁷O/g water), S_o is the signal of ¹⁷O at the start of the experiment, S_t(t) is the final concentration after the measurement period of metabolism from a linear fit of the rising signal over the calculation time, and f₁ is set to .77 (Herscovitch and Raichle, 1985).

The cerebral metabolic rate of oxygen consumption is calculated from the first 50 seconds of oxygen inhalation, after a 10 second delay. This delay reflects the time it takes for the ¹⁷O₂ gas to reach the tissue as described in the previous section.

Materials and Methods

Breathing circuit design

The precision delivery circuit used was developed for gas experiments to provide step changes in concentration of gas at the mouth with precise timing and minimal gas loss (Baumgardner et al., 2008). When used with large animals as in the experiments described here, respiratory rate is set to 6 breaths/min and a tidal volume of 18 mL times the weight in kg (i.e. 450mL for a 25kg pig). In these experiments, 1.2 times a single tidal volume of enriched $^{17}\text{O}_2$ was diluted with nitrogen to a final concentration of 20% enriched $^{17}\text{O}_2$ and 80% N_2 and then delivered over 6 breaths. Delivery begins at time 0 in all plots.

To briefly explain the design of the Precision Delivery Circuit, a representation of the system appears in Figure 2. The system connects from outside the magnet room through three sets of tubing to the endotracheal tube of a large animal subject. Pressure at the mouthpiece was measured by a transducer connected via 25 feet of 0.125" internal diameter tubing (Freescale Semiconductor, Chandler AZ, MPX2010DP). That pressure transducer reports to custom written software in LabView via a multifunction data acquisition device after amplification by a custom operational amplifier circuit. The computer then uses those pressure measurements to adjust inhalation and exhalation pump speeds, overcoming the resistance of 25 feet of .25 inch tubing. The inhalation line and exhalation line connect to large peristaltic pumps (Cole Parmer, Chicago IL, Masterflex I/P Digital Drive). The computer also controls valves (VICI Valco Instruments, Houston TX, Model C45) that select the input gas or output flow for recapture or to vent to room air. Gases are kept at ambient pressure in bags filled before the experiment with low loss circuits to ensure that any error during the experiment will not result in the loss of large volumes of pressurized $^{17}\text{O}_2$ gas.

Animal Experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee and followed the guidelines of the "Principles of laboratory animal care". Five male Yorkshire swine weighing from 19kg to 41kg (age 2–4 months) were used. Induction of anesthesia was performed by an initial IM injection of ketamine 22mg/kg, xylazine .025mg/kg, and atropine .05mg/kg and maintained with ketamine 60mg/kg/hr and diazepam 2mg/kg/hr. This regimen should not significantly affect brain metabolism. While ketamine has been shown to stimulate brain metabolism in certain animals (Hoffman et al., 1992), there is evidence in swine that this stimulation is canceled by benzodiazepines (Akeson et al., 1993). Fluid balance was maintained with normal saline at 2ml/kg/hr. Animal heart rate and oxygen saturation were monitored with a standard infrared pulse-oximeter and full oxygen saturation (98–100%) without fluctuation was required for the experiments to proceed. All experiments begin with T_1 -weighted sequence for localization and anatomical imaging of the brain.

Magnetic Resonance Imaging

Direct imaging was performed on a broadband-enabled 3T Siemens Trio scanner. Proton images were taken with the body coil and then without moving the pig, a custom built interface was connected to the scanner and interfaced with a home built 9cm surface coil tuned to 16.71MHz placed on top of the pig's head. Calibration of the utilized rectangular excitation pulse was performed by taking a number of gradient recalled echo images and maximizing signal-to-noise over the brain region. One ^{17}O image was taken in each plane. Parameters were as follows: repetition time (TR) 100ms, TE 1.8ms, field of view (FoV) 40×40cm, 64×64 matrix, bandwidth 200Hz/Pixel, asymmetric echo. Following that a series of pulse acquire spectra were taken with the same hard pulse over 15 minutes. Pulse acquire parameters were: TR 100ms, 256 points, two step phase cycling, 40kHz bandwidth, repeated 9000 times over 15 minutes.

Indirect imaging was performed on separate occasions on a 1.5T Siemens Sonata scanner. Images were taken with a 15cm vendor supplied surface coil placed on the head of the pig. Serial images during room air and $^{17}\text{O}_2$ delivery were acquired with a $T_{1\rho}$ -prepared single-shot, high flip angle, centrically encoded, fully-balanced gradient echo sequence reported shown in Figure 3. A more thorough treatment of this sequence and its use to detect mH_2^{17}O *in vivo* are presented in a separate manuscript (Mellon et al., 2008). Parameters were: TR 9.7ms, TE 4.7ms, slice thickness 6mm, FoV 200mm², 128×128 matrix, bandwidth 130Hz/Pixel, flip angle 180° (opening pulse 90°), spin locking amplitude 100Hz, spin locking time 75 or 200ms, fat saturation on, time per image 1.6 seconds, 2 second delay for T_1 recovery.

Arterial Blood Sampling and ^{17}O NMR spectroscopy of blood

An arterial catheter was placed under ultrasound guidance into the femoral artery in the large animal fluoroscopy suite. During imaging, approximately 2cc of blood was collected over 3–5 seconds into each Vacutainer Serum Separator Tube (Becton-Dickinson, #367983) at a rate of one sample each 10 seconds for the first 12 samples and then 60 seconds each for six more samples. Four control samples were taken before the experiment began. The tubes used are designed to reseal immediately after needle puncture. Their ability to remain sealed was tested by taking several tubes at random, creating a strong negative pressure inside the tube with a vacuum pump attached to a needle, and then repeated puncture with larger diameter needles than used for blood sampling. The pressure inside the tubes was checked after over a dozen punctures and had changed less than 10%. Storage of a sampling of tubes over a period of a week showed no repressurization over time.

A concern is that water ^{17}O will exchange with ambient CO_2 through bicarbonate ion, a very fast reaction when catalyzed by carbonic anhydrase as in whole blood (Mills and Urey, 1940). To minimize losses to the air at the time of analysis, serum was separated from blood by centrifugation soon after the experiment to remove the carbonic anhydrase from analyzed blood water. Because carbonic anhydrase is almost exclusively found in red blood cells, there is almost no carbonic anhydrase activity in serum (Meldrum and Roughton, 1933). Tubes were then frozen at -20°C and stored sealed. This procedure protects the blood because the tube is sealed, blood can mix only with the small amount of CO_2 (~450 parts per million) in the small amount of air (<1mL) drawn into the tube, a trivial source of mixing. A small amount of mixing can occur with the blood bicarbonate, but this reaction is extremely fast and occurs in the body regardless. As blood bicarbonate is <30mM (Recchia et al., 2000), this represents a trivial loss of ^{17}O . So when the serum is re-exposed to air, the reaction catalyst is missing, and completing the NMR measurement within 10 minutes of exposure to air suggests that the loss of ^{17}O is less than 5% during that time (Bitterman et al., 1988).

An 11.7T Bruker DMX400 Avance Spectrometer equipped with a $^1\text{H}/\text{X}$ nucleus decoupler probe tuned to ^{17}O was used to measure 1mL of each sample loaded into 5mm ID NMR tubes just before analysis. Spectra were recorded without lock at room temp. Five samples taken before the start of the experiment comprise the data point at time 0, assumed to be the natural abundance of H_2^{17}O , with a standard deviation of .17 $\mu\text{mol/g}$ serum. The parameters for ^{17}O spectroscopy were: TR 41ms, 4096 points, bandwidth 50kHz, 4000 averages, flip angle 90°.

Data Analysis

For the direct measurement of mH_2^{17}O production, the integrals of the pulse acquire spectra from 4 averages per data point (400ms each) were acquired over 15 minutes. The spectra at the start of $^{17}\text{O}_2$ delivery was taken to be the natural abundance H_2^{17}O signal (20.35 $\mu\text{mol/g}$), which served as the internal calibration for mH_2^{17}O concentration changes ($C_b(0)$ in Equation 16). The signal trace during $^{17}\text{O}_2$ delivery was fit linearly over 50 seconds beginning 10 seconds after the start of the $^{17}\text{O}_2$ pulse to generate the mH_2^{17}O production for Fig 1 in Table 2. The

slope and standard error of the linear fit was used in Equation 16 as $S_t(t)$. The plots of the direct signal change were temporally smoothed over 16 seconds before and after to show the trend in signal change.

For indirect measurement of $CMRO_2$, region of interest analyses were performed by segmenting each half of the pig brain without including ventricles based on anatomical contrast images and a pig brain anatomy atlas. The time course of each ROI in the indirect imaging was temporally smoothed over 16.2 seconds (4 points) before and after to filter high frequency noise. A linear fit was performed over 50 seconds approximately 10 seconds after the start of the $^{17}O_2$ pulse, and the slope of that line was used to estimate the signal change and fitting error per minute.

Results

Blood sampling was performed during scanning on two separate occasions. The delay between the start of $^{17}O_2$ delivery and the start of wash-in, defined as two successive points over the standard deviation of the measurement, is shown in Figure 4 to be about 90 seconds in the 40.8kg pig. A second experiment showed the delay to be on the order of 70 seconds in a 19.8kg pig (not shown). This verifies that recirculation of $mH_2^{17}O$ is minimal until at least 60 seconds after the start of $^{17}O_2$ delivery, and thus validates Equation 8 for this purpose.

Figure 5a–c shows direct ^{17}O images taken at 3 Tesla with a surface coil placed on the head of the pig in each plane to demonstrate the area examined by the time series spectra in Figure 5d. This is overlaid on ^1H images taken with the body coil. Figure 5d shows a time series plot derived from the integrals of ^{17}O spectra taken with the same coil configuration and excitation pulse.

The correlation of indirect and direct imaging is shown in Figure 6a. Figure 6b shows the time series plot of blood sampling in Figure 4 combined with direct imaging shown in Figure 5d.

Figure 7 shows an example image and metabolic map.

Figure 8 demonstrates the changes in $mH_2^{17}O$ post $^{17}O_2$ delivery during the first 60 seconds and shows the linear fit to the data points used to calculate $CMRO_2$. The slope of the signal change in half of the brain in one central slice of brain gives the hemispheric $mH_2^{17}O$ production and that is calculated for each hemisphere in Table 2 and converted to $CMRO_2$ measurements. The f values correspond to the fractional enrichment of ^{17}O delivered where .41 corresponds to delivery of 70% $^{17}O_2$ and .24 corresponds to delivery of 40% $^{17}O_2$. The sensitivity of the technique is reflected in the standard errors for each hemispheric measurement, which average to 36% per measurement. Taken together, these demonstrate that $CMRO_2$ can be calculated by the signal change of 1 minute of inhalation by the indirect technique at 1.5 Tesla.

Discussion

The $CMRO_2$ of pigs of comparable size using a similar continuous infusion ketamine and benzodiazepine anesthesia regimen has been estimated to be $1.63 \pm .19 \mu\text{mol/g/min}$ by ^{15}O PET (Poulsen et al., 1997). The spatial resolution of the measurements presented here is the same as those ^{15}O PET images without the need for an onsite cyclotron or radioactivity. Still, differences in pig $CMRO_2$ found in the literature vary significantly depending on the technique, anesthesia regimen, and size of pig used. One study averages $1.20 \pm .29 \mu\text{mol/g/min}$ and $1.15 \pm .46 \mu\text{mol/g/min}$ by radioactive and fluorescent microspheres respectively (Ehrlich et al., 2002). Another radioactive microsphere study in pigs of different ages found $CMRO_2$ ranging

from 1.30 to 1.75 $\mu\text{mol/g/min}$ (Ichord et al., 1991). The measurements made here compare favorably to these values.

While 3 Tesla scanners are relatively widespread clinically, lower fields have some advantages in the indirect imaging application. The J-coupling and chemical exchange interactions that lead to relaxation enhancement are field strength independent. As such, higher starting T_2 values for tissue make the absolute T_2 reduction by H_2^{17}O greater at lower field strengths. Also, lower tissue T_1 values allow for faster averaging of the spin-spin coupling based H_2^{17}O effect. Further, diminished magnetic susceptibility effects reduce the competing signal changes from cerebral blood and sequence artifacts. This is in contrast to direct ^{17}O measurements that benefit from ultra-high fields. As such direct measurements are typically performed at clinically unavailable field strengths. For all these reasons we chose a 1.5T scanner for our indirect studies.

Nevertheless, the major technical limitation of ^1H detected ^{17}O imaging is the relatively small effect of physiologic levels of ^{17}O on ^1H relaxation. We are confident that this problem can be overcome in human applications. The human brain is so much larger than pig brain that region of interest analyses should be more easily obtainable and more meaningful. The ^{15}O PET literature lends evidence for this claim. The CMRO_2 maps taken from humans (Langsjo et al., 2003) are more impressive than those taken from swine (Smith et al., 1998), and quantification can then be made regionally instead of hemispherically. As humans are far more cooperative than pigs and do not require anesthesia, human studies should be easier to perform. Further, as units of ^{17}O will cost less as more is produced, it is hoped that the cost of these techniques will be improved by increased interest and usage by the research community.

It is unclear if these techniques will be able to measure CMRO_2 in functional MRI due to the limited sensitivity of the technique and the competition from the flow and blood oxygen level dependent effects. Electrical stimulation of the paw in rats for example imparts a 19% increase in regional brain CMRO_2 (Mandeville et al., 1999) which is a small and very spatially localized change. Future improvements in these techniques may make this possible. One improvement would be the precise measurement of R_2 or $R_{1\rho}$ due to H_2^{17}O as discussed in the Theory. However, a more significant contribution to CMRO_2 measurement uncertainty is provided by physiologic and electronic noises, which are the primary contributors to the reported measurement standard errors. Nevertheless, the primary intent of our group is to develop these techniques for use in major disturbances in metabolism, such as ischemia, tumor, and neurodegeneration, where metabolism is greatly disturbed and cannot be studied by existing functional MRI techniques. Studies are underway to demonstrate this possibility, and future studies in humans should become easier due to the reasons listed above.

It must be noted that the equations used to quantify CMRO_2 have sources of potential error for absolute measurements. The value of the relaxation enhancement due to ^{17}O on ^1H , R_2 , for example is difficult to measure *in vivo* or with *ex vivo* tissues, and as such it may be imprecise. As well, R_2 may vary based on different tissue parameters such as grey or white matter or cerebral blood volume per voxel. It is also known that R_2 has a dependence on pH (Meiboom, 1961). This is a very minor concern for application in cerebral infarction, as one rat model shows overall tissue pH in hyperacute stroke leading to complete infarction to drop to a minimum of 6.6 (Nedergaard et al., 1991) and R_2 shown by Meiboom only varies about 10% over the pH interval 6.2–7.8. Also, since R_2 is reduced by pH extremes, further drops in pH would lead to an underestimation of mH_2^{17}O production. This serves to increase the probability that tissue infarction would be detected by future applications of this technology. The value of f , the tissue fractional enrichment of ^{17}O in the oxygen gas, chosen is based from a very simple, approximate model of inhalation appropriate for averaging over several breaths; however more sophisticated modeling could potentially be used. This factor has not been considered in the

estimation of CMRO₂ with ¹⁷O₂ previously because small animals have high respiratory rates and the slurring of the ¹⁷O fractional enrichment may be neglected because gas mixing in the lungs occurs much more quickly. Also, it becomes more reasonable to neglect f with longer time periods of inhalation where the fractional enrichment has had sufficient time for equilibration with the enriched ¹⁷O gas. Despite these absolute measurement considerations, relative CMRO₂ in the same subject or across subjects can be estimated using the current techniques.

The T_{1ρ}-prepared, centrically encoded, fully balanced spin echo sequence used in this study evolved from decoupling experiments in which high T_{1ρ} spin lock amplitudes (Rizi et al., 1998), off resonance spin-locking (Charagundla et al., 1998), or ¹⁷O decoupling (Reddy et al., 1996) are used to mitigate the ¹H-¹⁷O J-coupling interaction. This sequence improves the temporal resolution for these decoupling techniques significantly by allowing them to be performed with a fast single shot imaging readout. This brings down the temporal resolution from at least 10 seconds per image with turbo spin echo based readouts to about 3.5 seconds with the current sequence and parameters. The small spin lock amplitude, 100Hz, used here gives extremely similar R_{1ρ} constants to measured R₂ in the case of ¹⁷O (Reddy et al., 1995; Stolpen et al., 1997) without visible artifact or effect of macroscopic background field gradients. This study was performed with a body coil transmitter and surface receiver, which is suggested for the high homogeneity of the body coil for spin-locking. Decoupling of the ¹H-¹⁷O interaction can be performed with high spin lock amplitudes as well. While decoupling is not performed in this study due to implementation difficulties and specific absorption rate concerns on clinical scanners, decoupling experiments similar to those previously conducted (Reddy et al., 1996; Reddy et al., 1995) can be performed using the sequence and delivery techniques presented here. Also, it may be possible that the same estimates could be made with fast T₂ weighted sequences such as spin echo echo planar imaging or single shot turbo spin echo methods. In particular, spin echo echo planar imaging presents the possibility of obtaining 3D metabolic images. However, caution must be exercised using sequences that rapidly oscillate their gradients, as any possible distortion or artifact may mask the small proton signal change generated by physiologic short-term mH₂¹⁷O production. T_{1ρ} imaging with a spin-echo readout, as shown here, enables us to measure the small changes due to mH₂¹⁷O that may not be seen with other sequences due to artifacts that may obscure small signal changes in long T₂-weighted images or in echo planar imaging.

While it is recognized that the anesthesia regimen may change the vascular recirculation of mH₂¹⁷O as opposed to awake humans, multiple ¹⁵O PET studies have modeled the time course of recirculation for metabolic water using similar time courses with a single breath hold in humans and these have also made CMRO₂ measurements claiming recirculation during the first minute to be negligible (Meyer et al., 1987; Ohta et al., 1992). Indeed, the measurements made here and the modeling used for CMRO₂ measurement is very similar to ¹⁵O experiments performed in humans (Mintun et al., 1984). In that study, 40 seconds were used to measure CMRO₂ after a 20 second delay after start of inhalation. Recirculation of H₂¹⁵O was measured and found to be minimal during that time. The error in CMRO₂ calculation ignoring wash-in was simulated to be less than 15% for very critically low values of oxygen extraction fraction (<.15), and less than 5% for typical oxygen extraction fractions (>.3). With the current data and the PET data taken together, it seems the recirculation delay measured in swine is comparable to humans, and as such, anesthesia or size of swine is not thought to be a significant factor for the kinetics of mH₂¹⁷O recirculation. If anything, these experiments should become easier to perform in humans who have larger brains and do not require anesthesia for imaging.

Another difference between the current study and many short time course PET studies is that for delivery PET studies frequently use a single tidal volume inhalation of tracer with a breath hold. We do not employ this strategy here for several reasons. First, any fluctuation in blood

oxygen content results in a change in MRI signal (Bulte et al., 2007; Chiarelli et al., 2007). A single bolus of 100% oxygen after breathing room air will cause an increase in MR signal that will interfere with the measurement (suggested by above references and demonstrated by the authors, data not shown). It is possible to have the subject breathe 100% oxygen for a period of time to stabilize the MR signal before $^{17}\text{O}_2$ delivery and this has been done by us with similar mH_2^{17}O observations. However, modeling indicates that this wastes much more expensive $^{17}\text{O}_2$ gas by exhalation of oxygen unused by the lungs than the method presented here. This is because hemoglobin is fully saturated past ~15% inhaled O_2 at 1 atm pressure and as such most of the oxygen in a 100% oxygen inhalation will not be absorbed. A large single breath of $^{17}\text{O}_2$ could potentially be held longer to decrease the amount of exhaled ^{17}O , however this risks even small amounts of hemoglobin desaturation which will artificially enhance the ^{17}O effect by $T_2^*/T_2/T_{1\rho}$ reduction by paramagnetic venous deoxyhemoglobin. Still, with careful bolusing and hemoglobin saturation accounting, a single breath MRI method could be obtained that would use approximately the same amount of $^{17}\text{O}_2$ used here.

In conclusion, we have demonstrated the feasibility of measuring CMRO_2 in large animals on clinical scanners using a single tidal volume of $^{17}\text{O}_2$ gas delivered with a precision delivery circuit. By minimizing gas required for CMRO_2 measurements, employing a large animal model, and utilizing clinical hardware, this represents a crucial first step towards the translation of small animal $^{17}\text{O}_2$ studies to humans. A simple model of lung mixing and delivery to tissues is presented. Arterial blood sampling and analysis of mH_2^{17}O content shows the time when recirculation begins to be 60–80 seconds—ample time for fast imaging techniques to obtain numerous images for CMRO_2 estimation. High temporal resolution indirect and direct imaging is correlated to show similar results for the estimation of CMRO_2 non-invasively during those 60 seconds. Work is already underway to detect experimental derangements of metabolism. It is hoped that future studies will detect metabolic derangements in humans using these techniques. For indirect imaging only a clinical 1.5T scanner with standard hardware and an easily programmable pulse sequence is necessary.

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Figure 1.

Plotted is a step function simulation of brain tissue ^{17}O fractional enrichment in oxygen gas when 15 breaths of 20% oxygen at 70% ^{17}O enrichment is given to a pig with the parameters in Table 1. The time of delivery for the CMRO₂ estimates in this paper is 6 breaths (1 minute), but data out to 15 breaths is shown here to demonstrate the time to asymptote at the delivered enrichment. Delivery of gas begins at time 0, but a 10 second delay is given for breathing circuit delay, mixing time, and uptake and transport time of oxygen to the brain. The average of the area shaded in grey under the step function is used as f for computing CMRO₂ introduced in Equation 9.

Precision Delivery Circuit

Figure 2.

A schematic for the custom, low-loss, precision delivery breathing circuit used to deliver ¹⁷O₂ described in the text. The system can be split into three main parts labeled by color and component number: inhalation (green, 1), pressure sensing (blue, 2), and exhalation (red, 3). Label 1a refers to the inhalation line of the circuit, which has gas delivered by a pressure feedback controlled peristaltic pump (1b). The pumped gas is selected by a computer controlled switch valve (1c) and in these experiments the gas was one of air, oxygen, or ¹⁷O₂ enriched gas mixture. The label 2a refers to the smaller diameter pressure sensing line, which feeds pressure at the airway to a pressure transducer (2b) that reports to a laptop (2c). The laptop monitors the pressure and controls the pumps. Also, user input to the laptop changes the gas source and destination via the switching valves. Label 3a refers to the exhalation line, through

which gas is removed from the animal's respiratory system by a separate peristaltic pump (3b). The destination of the exhaled gas is selected by a computer controlled switching valve (3c) to either the room or through a CO₂ scrubber (3d) to an exhaled gas sampling bag (3e).

Figure 3.
The spin lock prepared balanced steady state free precession readout used to estimate CMRO₂.

Figure 4.

Shown are the results of a serial arterial blood sampling experiment during and after 1 minute of $^{17}\text{O}_2$ delivery. Delivery of $^{17}\text{O}_2$ as 80% N_2 /20% O_2 (70% ^{17}O enriched) begins at time 0 seconds and ends at 60 seconds as in all the following figures. Serum concentration of H_2^{17}O was calculated by integrating the H_2^{17}O peak obtained by ^{17}O spectroscopy of the blood samples taken at the given time points. The solid line is a 2 point moving average of the data points. The first data point is an average of 5 samples taken before the start of $^{17}\text{O}_2$ delivery, assumed to be $20.35 \mu\text{mol H}_2^{17}\text{O/g water}$. The error bar is the standard deviation of those 5 points of $0.17^3 \mu\text{mol/g}$.

Figure 5.

a.–c. Three colorscaled ^{17}O images taken before $^{17}\text{O}_2$ inhalation (natural abundance) are overlaid over grayscale proton images from the same locations (Tra-Transverse, Sag-Sagittal, Cor-Coronal). A color bar on the right shows the linear relative ^{17}O signal. The differences in signal are mostly due to the surface coil sensitivity profile and not due to any differences in ^{17}O content except in large difference areas such as the air spaces. This is presented to show the area that H_2^{17}O is averaged over in the measurements for fig 1, also in Figure 6a. d. The same excitation pulse as in a–c was used to take serial pulse acquire spectra and the H_2^{17}O peak integrals are plotted over time and converted by Equation 16 to indicate $\mu\text{mol H}_2^{17}\text{O/g}$ water.

Figure 6.

a. The metabolic water change measured by separate experiments using direct ¹⁷O spectroscopy and proton-detected H₂¹⁷O is presented to show the similarity *in vivo*. The direct ¹⁷O spectroscopy plot is used to derive the CMRO₂ for pig 1 (1.19 ± 0.43). The indirect ¹⁷O trace is the result of left hemisphere ROI analysis (excluding ventricles) from pig 2. Excellent agreement between the indirect and direct signals verifies the specificity of the current methods. The indirect signal is converted to concentration of mH₂¹⁷O by Equation 14.

b. Demonstrated is the rise in metabolic water from brain without a concurrent rise in recirculated metabolic water. The difference between the two lines indicates the CMRO₂. Since the difference between them is so great, the recirculation can be effectively ignored.

Figure 7.

a. An example image taken during the time series of fig 5. Areas of enhancement correspond to regions at least partially filled with cerebral spinal fluid. b. A pixel-by-pixel linear fit for fig 5 during the delivery of $^{17}\text{O}_2$ converted to CMRO_2 per pixel, cropped to fit the brain. The smoothing function used is a 1 pixel Gaussian, leading to a final nominal resolution of $3.6 \times 3.6 \times 6\text{mm}$ at full width half maximum. The largest change is seen around the edge of the brain representing brain cortex and in the brainstem, both areas expected to have the highest metabolism. Some noise is seen, however very low metabolism is seen in high fluid regions such as in the ventricles. Cropping of the map to brain was performed because of the low signal-to-noise obtained from the shorter T_2 muscle surrounding the brain. The skin and skull between the brain and the coil is estimated to have very low metabolism (Martin and Fuhrman, 1955), and from those nothing could be measured.

Figure 8.

Examples of hemispheric changes (Right and Left) in water production used to generate CMRO₂ estimates in the swine listed in Table 2. The signal from each ROI at each time point was converted to units of μmol metabolically produced water per g brain. This is done by inputting the signal to Equation 15 leaving out the factor of 2. The straight lines are the linear fits to the individual points. It is the linear change in signal that is used to generate Table 2. The amount of deviation over that time represents the standard error of the measurement.

Table 1 Parameters for simulation of ^{17}O fractional enrichment absorbed during the first minute of inhalation

	Description	Value (Ref)	Unit
TV	Tidal Volume	18	mL/kg
fiO ₂	Inhaled fraction of oxygen	0.20	
α	Fractional enrichment of inhaled $^{17}\text{O}_2$	0.7	
FRC	Functional Residual Capacity	50 (*)	mL/kg
VO ₂	Whole body O ₂ consumption	5 (**)	mL/kg/min
RR	Respiratory Rate	6	breaths/min

* References (Mutoh et al., 1992)

** (McKirnan et al., 1986; Willford et al., 1986)

Table 2

Estimated cerebral metabolic rates of oxygen consumption (CMRO2)

Direct ^{17}O	f	f_I	CMRO2 $\mu\text{mol/g/min}$	CMRO2 error
Pig 1 Whole Brain	.41	.77	1.19	0.43
Indirect ^{17}O	TSL			
Pig 2 Left Hemisphere	.24	205	1.03	0.33
Pig 2 Right Hemisphere	.24	205	1.30	0.26
Pig 3 Left Hemisphere	.24	205	1.49	0.39
Pig 3 Right Hemisphere	.24	205	1.63	0.62
Pig 4 Left Hemisphere	.41	80	0.88	0.52
Pig 4 Right Hemisphere	.41	80	1.46	0.7
Pig 5 Left Hemisphere	.41	80	1.34	0.33
Pig 5 Right Hemisphere	.41	80	1.54	0.59
Indirect Average				1.33 ± 0.26