Evolving techniques for the investigation of muscle bioenergetics and oxygenation
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Abstract
Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are both powerful, non-invasive methodologies and, as such, offer great potential to investigate both human biochemistry and human physiology, and ultimately to contribute significantly to the field of medicine. Consequently there has been much effort devoted to fostering the evolution of these methodologies into distinct and applicable techniques. Here we will highlight several MRI and MRS techniques for the assessment of human biochemistry and physiology that ultimately may provide useful clinical assessments and diagnoses of various muscular and cardiovascular pathologies. Specifically, the evolving techniques that will be discussed are: (1) $^1$H MRS of myoglobin to assess the intracellular partial pressure of oxygen ($P_{\text{O}_2}$), (2) $^{31}$P MRS to assess metabolic capacity, and (3) the combination of $^{31}$P chemical shift imaging to assess local metabolic demand (oxygen uptake; $\dot{V}_{\text{O}_2}$) with arterial spin labelling to assess local perfusion (blood flow; $\dot{Q}$), in an effort to characterize the elusive spatial matching of skeletal muscle ($\dot{Q}/\dot{V}_{\text{O}_2}$).

Introduction
Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), both applications of NMR, are remarkably versatile methodologies. As such, since the discovery of NMR in 1946 [1,2], both MRI and MRS have played an important role in both physics and analytical and structural chemistry. However, in more recent years the application of these methodologies to biochemistry, physiology and medicine has had a tremendous impact in clinical and research settings. NMR signals can be obtained from all magnetic nuclei, specifically from all nuclei with non-zero spin. Currently the nuclei of most interest in physiology and medicine are $^1$H, $^{13}$C and $^{31}$P. MRI and MRS use essentially the same basic equipment to elicit and collect an NMR signal [a high-field magnet, a radio frequency (RF) coil, a RF generator/receiver and a computer], but differ in that MRI provides spatial separation of data (producing an image), whereas MRS results in the separation of chemical species (producing spectra representative of concentrations).

The purpose of this paper is to bring into focus the continued development of several MRI and MRS techniques for the assessment of human biochemistry and physiology that ultimately may provide useful clinical assessments and diagnoses of various muscular and cardiovascular pathologies. Specifically, the evolving techniques that will be discussed are: (1) $^1$H MRS of myoglobin (Mb) to assess the intracellular partial pressure of oxygen ($P_{\text{O}_2}$), (2) $^{31}$P MRS to assess metabolic capacity, and (3) the combination of $^{31}$P chemical shift imaging (CSI) to assess local metabolic demand (oxygen uptake; $\dot{V}_{\text{O}_2}$) with arterial spin labelling (ASL) to assess local perfusion (blood flow; $\dot{Q}$) in an effort to attain the elusive assessment of the spatial matching of $\dot{Q}/\dot{V}_{\text{O}_2}$.

Methods
Exercise modalities
Two exercise modalities have been employed as part of these evolving techniques. Knee-extensor exercise was utilized during the $^1$H MRS studies of Mb desaturation, while plantar flexion exercise was used during both the $^{31}$P MRS studies and the combination of $^{31}$P CSI and ASL during exercise. For plantar flexion exercise, subjects performed constant-load submaximal plantar flexion (contraction frequency 1 Hz) while lying supine in a superconducting 1.5 T magnet. For single-leg knee-extensor exercise, subjects lay supine on a padded bed with the knee-extensor ergometer placed in front of them (illustrated in [3]). The contraction frequency was 1 Hz.

Key words: arterial spin labelling, chemical shift imaging, exercise, myoglobin, perfusion.
Abbreviations used: ASL, arterial spin labelling; CSI, chemical shift imaging; Mb, myoglobin; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PCr, phosphocreatine; $P_{\text{O}_2}$, partial pressure of oxygen; $\dot{Q}$, blood flow; RF, radio frequency; $\dot{V}_{\text{O}_2}$, oxygen uptake.

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All studies reported were approved by the UCSD and University of Pennsylvania Human Subjects Committees and all subjects gave informed consent to participation.

Determination of intracellular \( P_{O_2} \)

Spectra were collected from the quadriceps muscle region below the 7 cm-diam. surface coil double-tuned to proton (85.45 MHz) and phosphorous (34.59 MHz) placed over the rectus femoris portion of the quadriceps group [4]. Details of the theory behind oxygen-sensitive Mb signals have been published previously [3,5]. Briefly, the haem iron exhibits oxygen-dependent spin states that in turn influence nearby protons. The N-ο proton on proximal histidine F8, one the ligands coordinated to the iron, is particularly sensitive to these changes. When oxygen is bound to the active site, the resonance of this proton is hidden beneath the dominant water signal. However, when Mb becomes deoxygenated, changes in the iron spin state shift this peak to a temperature-dependent position that is clearly distinct from all other resonances.

Under conditions of cuff ischaemia, intramuscular \( O_2 \) is depleted within approx. 8 min of occlusion [6]. Therefore the plateaued signals obtained during the 10th and 12th min of cuff occlusion (270 mmHg) represent complete deoxygenation of Mb; these were used to estimate total Mb content within the muscle. During exercise the fractional deoxyMb (fdeoxyMb) was determined by normalizing signal areas to the average signal obtained during the last minutes of cuff ischaemia. The conversion from fdeoxyMb to \( P_{O_2} \) values was then calculated from the oxygen-binding curve for Mb:

\[
P_{O_2} = \frac{1}{f_{MbO_2}} \cdot \frac{P_{90}}{f_{deoxyMb}}
\]

where \( f_{MbO_2} \) is the fraction of Mb that is oxygenated and \( P_{90} \) is the \( O_2 \) pressure when 50% of the Mb-binding sites are bound with \( O_2 \). A temperature-dependent Mb half-saturation (\( P_{90} \)) of 3.2 mmHg was used [7].

Skeletal muscle \( P_{O_2} \) during single-leg knee-extensor exercise was determined from blood samples taken from the radial artery and femoral vein in conjunction with the measurement of muscle blood flow by the thermodilution technique, as reported previously [8,9]. This protocol was repeated in room air (21% \( O_2 \)) and under conditions of hypoxia (12% \( O_2 \)) and hyperoxia (100% \( O_2 \)).

\( ^{31}P \) MRS

MRS was performed using a clinical 1.5 T General Electric Signa system (5.4.2 version) operating at 25.86 MHz for \( ^{31}P \). \( ^{31}P \) MRS data were acquired with a dual-frequency flexible array spectroscopy coil (Medical Advances Inc.) placed around the calf at its maximum diameter. The phosphorus coil was an 11.5 cm square centred between two 14 cm x 15.5 cm Helmholtz-type proton coils. Magnetic field homogeneity was optimized by shimming on the proton signal from tissue water, and the \( ^{31}P \) MRS signal was optimized by prescan transmitter gain adjustment. A 500 μs hard pulse was used for signal excitation. The spectral width was 2500 Hz, and data were acquired continuously for 13 min, with a single free induction decay acquired every 4 s. Thus 195 free induction decays were acquired during the 2 min rest period, 6 min of plantar flexion exercise and 5 min of recovery. This protocol was repeated in room air (21% \( O_2 \)) and under conditions of hypoxia (12% \( O_2 \)) and hyperoxia (100% \( O_2 \)).

\( ^{31}P \) CSI

Two series of \( ^{31}P \) CSI studies were performed using a 1.5 T MRI scanner (GE Medical Systems, Milwaukee, WI, U.S.A.), one at rest and one during 10 min of steady-state plantar flexion exercise. A dual-tuned \( ^{31}P/^{1}H \) flexible coil (Medical Advances) was used to obtain \( ^{1}H \) axial images (to confirm anatomical localization) and \( ^{31}P \) CSI data. \( ^{31}P \) CSI data were acquired using the commercially available pulse sequence provided by GE Spectroscopy Research Accessory, in a similar fashion to previous studies [10]. Specifically, an RF pulse to excite magnetization within a slice was applied in the presence of a gradient. In order to obtain both spectral and spatial information, phase-encoding gradients were applied before the data readout. The spatial resolution is determined by the number of phase-encoding steps and by the field of view. A 14 cm field of view was used with an acquisition matrix size of 14 x 14 phase-encoding steps and a slice thickness of 1 cm. This resulted in an in-plane resolution of 1 cm² and a 1 ml volume of tissue in each voxel. B₀ homogeneity was adjusted for each subject using the autoshim capabilities of the scanner, with the transmitter frequency on resonance for the water signal. The transmitter frequency was switched to phosphorus and adjusted to be on resonance for phosphocreatine (PCr) to ensure that there was no misregistration between the anatomical images and the CSI data.
set due to chemical shift errors (these anatomical images, using pixel registration, ensure an accurate combination of the CSI and perfusion data sets). The difference spectrum (rest and exercise) for each voxel was determined off-line using a Silicon Graphics INDIGO equipped with SAGE (GE Medical Systems).

**Perfusion imaging using ASL**

All images were acquired on a standard 1.5 T clinical imaging system (GE Medical Systems) fitted with a local gradient knee coil of our own design [11] and built in our laboratory [12]. The detailed methodology of the ASL technique used to measure muscle perfusion has been published previously [13]. Briefly, imaging was performed using a modified version of continuous ASL [14,15], in which a short delay is inserted between inversion and image acquisition to reduce errors due to the spatial variations in the transit delay [16]. Alternating tag and control images were acquired every 5 s at a single location (repetition time = 5 s) in the axial plane with a 32 cm field-of-view, a matrix size of 64 x 64, and a slice thickness of 1 cm. The bandwidth was 125 kHz and the echo spacing was 624 µs. Sampling was not performed on the gradient ramps. The gap between the inversion region and the imaging slice was 3 cm. The delay, \( t_o \), between the end of the tag and image acquisition was 800 ms, the echo time was 20 ms, and the duration of the tag (\( t_t \)) was 1.3 s. A repetition time of 5000 ms was used in order to allow a time of 2.8 s between image acquisition and the subsequent inversion pulse for the subject to exercise. A total of 86 images were acquired in each experiment, with the exercise protocol (starting with the rest condition) beginning after the third image. The first two images were collected in order to equilibrate the magnetization, and were not analyzed. The control image was acquired by applying an off-resonance RF excitation pulse, in the presence of a gradient, on the opposite side of the imaging slice from the inversion slice. This design was similar to that used in the original implementation of continuous ASL in order to control for magnetization transfer effects [15].

**Results and discussion**

**'H MRS of Mb**

In combination, 'H MRS to determine Mb saturation, an endogenous probe of tissue oxygenation [6] and the functionally isolated human quadriceps muscle model [17] have provided the opportunity to study the relationships between intracellular and intravascular events in humans. Utilizing these techniques, we have revealed the relationship between skeletal muscle \( \dot{V}O_{2\text{max}} \) and intracellular \( PO_2 \) under conditions of hypoxia (12% \( O_2 \)), normoxia (21% \( O_2 \)) and hyperoxia (100% \( O_2 \)) (Figure 1B). The significant increase in \( \dot{V}O_{2\text{max}} \) associated with both an increase in \( O_2 \) delivery and the \( O_2 \) gradient from blood to cell support the theory that \( O_2 \) supply plays an important role in determining \( \dot{V}O_{2\text{max}} \) in trained skeletal muscle [18]. However, perhaps the most novel observation here is that, unlike the proportional linear increase in \( \dot{V}O_{2\text{max}} \) that occurs with an increase inMb \( PO_2 \) when moving from hypoxia to normoxia (including a hypothetical point at the origin), hyperoxia increased \( \dot{V}O_{2\text{max}} \) relatively less than Mb \( PO_2 \), suggesting that at this point the capacity to utilize \( O_2 \) (maximal mitochondrial capacity) is starting to play a role in this condition (Figure 1B).

This hyperbolic relationship, originating from the origin, between \( PO_2 \) and cellular respiration is in agreement with data described previously by Wilson et al. [19] in kidney cells (Figure 1A). We again [3], now with more conclusive data, suggest that these findings may represent the hyperbolic relationship between in vivo muscle \( \dot{V}O_2 \) and intracellular \( PO_2 \), supporting the concept that the maximal respiratory rate (i.e. \( \dot{V}O_{2\text{max}} \)) is normally limited by \( O_2 \) supply (Figure 1B).

**^31P MRS and varied \( O_2 \) availability**

PCr recovery is a useful measure of skeletal muscle oxidative capacity, and is dependent on mitochondrial respiratory function [20,21]. A further advantage of this measurement is that PCr recovery does not require a correction for active muscle mass [22,23], and is independent of the work level [24], provided that muscle intracellular pH does not fall severely [25]. Thus measurement of PCr recovery has proven useful in determining the oxidative capacity of skeletal muscle in a variety of conditions [26-28]. However, while providing an accurate index of skeletal muscle oxidative metabolism, this measurement does not allow differentiation between limitations caused by \( O_2 \) supply and those resulting from \( O_2 \) demand.

The assessment of an individual's response to manipulations of \( O_2 \) availability changes the measurement of PCr recovery time from a subjective to an objective evaluation of the limits to metabolic capacity (Figure 2). Thus, as discussed
previously [29], PCr recovery data are clearly indicative of both muscle $\dot{V}O_{2\text{max}}$ and muscle oxidative capacity. Earlier studies have illustrated a strong relationship between $O_2$ supply and skeletal muscle oxidative capacity during maximal exercise in trained subjects [30,31]. However, in sedentary subjects, maximal oxidative rate appears to be determined by mitochondrial capacity and not $O_2$ supply [32,33]. The data presented in Figure 2 illustrate the dependence on $O_2$ supply of PCr recovery in exercise-trained and active subjects, but not in sedentary subjects or those with a metabolic abnormality, while breathing a hyperoxic gas, consistent with the above findings. The rate constants of both the sedentary and active subjects were lowered while breathing 10% $O_2$, suggesting that the reduced arterial $P_{O_2}$ had influenced the rate of PCr recovery in each population. However, the data of Richardson et al. [32] showed that when sedentary subjects (with an activity profile similar to those examined in the present study) breathed 12% $O_2$, no effect on maximal oxidative rate was observed. This suggests that for sedentary subjects a 'critical $P_{O_2}$' may exist between breathing 10% and 12% $O_2$ where PCr recovery (and $P_{O_2\text{max}}$) would be unaffected by the fraction of inspired $O_2$. Hence the current technique ($^3$P MRS coupled with altered $O_2$ availability) appears useful to distinguish

**Figure 2**

**PCr recovery time (t) data**

Data were obtained using a $^3$P MRS protocol which combined variations in $O_2$ availability with plantar flexion exercise to determine if limitations to exercise are the result of inadequate $O_2$ supply or of the metabolic processes within the muscle itself [29]. * denotes a statistical difference between the other levels of $O_2$ availability as determined by repeated measures ANOVA.
Use of combined CSI and ASL data to measure local matching of \( \dot{Q}/\dot{V}_O_2 \) in human skeletal muscle in vivo

Upper panel: skeletal muscle perfusion image of the left calf muscle, collected using the ASL technique. The colour scale, from blue to red, represents variations in perfusion from low to high. Lower panel: PCr data collected by CSI during submaximal plantar flexion exercise with the left leg. This method allows the acquisition of comparable phosphorus spectra from multiple voxels (1 cm\(^3\)) simultaneously (A-M). The magenta-coloured PCr spectra were collected under resting conditions, while the blue spectra were obtained during exercise.

The importance of the spatial matching of skeletal muscle \( \dot{Q}/\dot{V}_O_2 \) is often cited, but this has been an elusive measurement to obtain. The ability to measure \( \dot{Q}/\dot{V}_O_2 \) is essential in order to examine and truly understand skeletal muscle function and dysfunction during the challenge of muscular work. For example, taken from one of our specific areas of interest (\( O_2 \) delivery and utilization during exercise), the finding that maximal \( O_2 \) extraction in exercising muscle is limited, such that the effluent venous \( O_2 \) content never falls to zero, has been interpreted as evidence of diffusion-limited \( O_2 \) supply [30,34–36]. However, this finding can be explained equally well by the existence of \( \dot{Q}/\dot{V}_O_2 \) inhomogeneity within an exercising muscle [37,38]. Indeed, there is considerable experimental evidence that perfusion heterogeneity in this scenario does exist [39,40], but each of these studies documented blood flow heterogeneity with respect to tissue volume, not \( \dot{V}_O_2 \). Consequently, the diffusion-limited component of \( O_2 \) transport has continued to be accepted, based upon the assumption that the heterogeneity of \( \dot{Q} \)/volume is matched equally by non-homogeneous metabolic \( \dot{V}_O_2 \)/volume. Thus, in areas where there is low \( \dot{Q} \), there may be low \( \dot{V}_O_2 \), and vice versa.

Figure 3 illustrates typical CSI and ASL data that are combined within 1 cm\(^3\) voxels to create a novel method for assessing the local matching of \( \dot{Q}/\dot{V}_O_2 \) in human skeletal muscle in vivo [41]. The need for such an assessment has been recognized for a significant period of time, but progress has been hampered by the methodological complexities of data collection. Utilizing this method, we have recorded considerable heterogeneity in both muscle \( \dot{Q} \) and muscle \( \dot{V}_O_2 \) during steady-state exercise, with no correlation between these two variables [41]. However, it is important to recognize that these data represent a methodological advance, and do not definitively characterize the relationship between \( \dot{Q} \) and \( \dot{V}_O_2 \) in exercising human skeletal muscle. This novel, non-invasive MRI method may ultimately provide such an assessment in both health and disease.

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References

Exertional oxygen uptake kinetics: a stamen of stamina?

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Abstract

The fundamental pulmonary $O_2$ uptake ($V_{O_2}$) response to moderate, constant-load exercise can be characterized as $(d\dot{V}O_2/dt)\cdot (d\dot{V}O_2) = \Delta V_{O_2}$, where $\Delta V_{O_2}$ is the steady-state response, and $t$ is the time constant, with the $V_{O_2}$ kinetics reflecting intramuscular $O_2$ uptake ($Q_{O_2}$) kinetics, to within 10%. The role of phosphocreatine (PCr) turnover in $Q_{O_2}$ control can be explored using $^{31}$P-MR spectroscopy, simultaneously with $V_{O_2}$. Although $t\dot{V}_{O_2}$ and $t^{PCr}$ vary widely among subjects (approx. 20–65 s), they are not significantly different from each other, either at the on- or off-