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# SIMULATION OF Mb/Hb IN NIRS AND OXYGEN GRADIENT IN THE HUMAN AND CANINE SKELETAL MUSCLES USING H-NMR AND NIRS

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## 1. INTRODUCTION

There has been great interest in the role of the oxygen carrying functions of myoglobin (Mb) and hemoglobin (Hb) in the skeletal muscles[1] which are thought to be associated with muscle performance[2], fiber types[3] and capillary density[4], indicating proportional use of oxygen carrying functions with oxygen demands. Mb and Hb have been shown to directly influence capillary, extra cellular and intracellular $PO_2$[5-6] to various degrees in different animals and organs[7-8]. Canine Gastrocnemius muscles consist of mostly fatigue resistant fibers and have a very high capillary density and high myoglobin[9], compared to human skeletal muscles[10]. They had a greater endurance to Mb desaturation in our pilot study, while human muscle exhibited about 50% of desaturation in the light exercise[11-12], and ischemia[13] using $^1$H-NMR. In the $^1$H-NMR, the deoxy Hb signal is shifted about 3 ppm from the Mb peak; however because of low visibility of the Hb signal, the contamination of Hb is negligible[14]. On the other hand, Near Infrared Spectroscopy (NIRS) can not distinguish between Hb and Mb because of their overlapped absorbance characteristics[15]. Thus the $^1$H-NMR Mb determination is ideal to help distinguish Hb and Mb in the NIRS signal by simulating Hb/Mb contribution to study oxygen gradients between capillary and myocytes. We have demonstrated possible Hb/Mb ratio as well as $PO_2$ in the capillary and myocytes in dogs and humans.

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## 2. MATERIALS AND METHODS

The canine and the human protocols were approved by the University of Pennsylvania laboratory animal research committee. Four mongrel dogs (ca. 10kg) were anesthetized with pentobarbital, and the Achilles tendon was tied to a string gauge, the force measurement. The calf muscle was stimulated at a sciatic nerve to yield a submaximal force development. After obtaining a resting period baseline, animals received submaximal stimulation with 10% hypoxia for 3-6 minutes. Thereafter, the FiO2 was gradually reduced to 0% anoxia while stimulation was maintained. In the human study, the arm was cuffed about 10 minutes either in the magnet for the NMR study, or outside the magnet for the NIRS measurement. The forearm was positioned at the same level as the heart to avoid blood volume changes due to a static pressure effect.

The NMR and NIRS data acquisitions were simultaneously carried out in a 40 cm bore 2.8 Tesla magnet in the dog study. The pulse sequence used for $^1$HNMR was similar to that reported previously[13]. A frequency domain NIRS (PM200, NIM Inc., Philadelphia) was used to measure HB + Mb signals. In brief, absorption coefficients of two wavelengths were obtained and used to calculate tissue oxygen saturation in the dog study. In the human study, CW NIRS imager using LED was used (NIM Inc.), and absolute saturation was calibrated based on the resting value of 50%[15] and 0% at the end of cuff ischemia.

Simulation of the Hb/Mb ratio in the NIRS was carried out to predict apparent Hb saturation using Hb and Mb $P_{50}$ of 26.6, and 3.2 torr[16], and Hills coefficients of 2.7 and 1 respectively. Molar equivalent of Hb, Mb for optical absorption coefficients in our wavelengths is 4:1. NIRS measures changes in oxygen saturation in the oxygen transferable blood volume during the experiments. This is the compartment of our interest, and we assumed that saturation ranged from 50% at rest[15] to 0% at the end of each experiment, when there is no longer oxygen available (anoxia). The resting myocyte $PO_2$ ($P_{myo}O_2$) is predicted as 18 torr[17]. The apparent Hb saturation, $S_{Hb}O_2$ is described as;

$$S_{Hb}O_2 = (1/(4\, f_{Hb}) + 3/4) \cdot S_{NIR}O_2 - (1/(4\, f_{Hb}) - 1/4) \cdot S_{Mb}O_2 \quad (1)$$

Where $S_{NIR}O_2$, $S_{Mb}O_2$ are saturations obtained from NIRS and NMR experimentally. $f_{Hb}$, $f_{Mb}$, are fractions of Hb and Mb ($f_{Hb} + f_{Mb} = 1$), assumed in the NIRS saturation signals respectively. The factors on the parameters in the Eq 1. came from translation of oxygen capacity from molar equivalent.

## 3. RESULTS

### 3.1. Human Cuff Ischemia Study with Simulation

From the measurements and $^1$H-NMR and NIRS, we plotted the Mb and Mb+Hb saturation curve over time (Figure 1A). There was an earlier and greater deoxygenation seen in the NIRS in the first 3 minutes to 70%, while the $^1$H-NMR signal had only 25% desaturation. After 3 to 6 minutes, there was a delayed rapid phase of desaturation in the $^1$H-NMR signal to 85%, while NIRS desaturated slightly by 25% at that time. From the timing differences of fast phases in NIRS and NMR, we can imply roughly that NIRS is more sensitive to the Hb, which desaturated faster, while the Mb desaturation comes after 3 minutes.