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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.

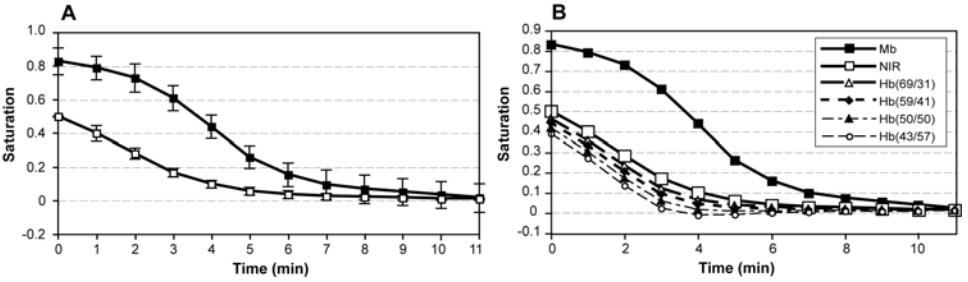

Figure 1. A; the time course of ^{1}H-NMR (closed rectangle) and NIRS (open rectangle) saturations during the arm cuff ischemia. Error bars indicate standard error. B; Saturations of Hb obtained from simulating $_{Hb}/f_{Mb}$ ratio from 100/0 (raw NIRS) to 47/57 (shown by the dotted lines), together with Mb saturation from NMR.

We then simulated the contribution of the Hb/Mb in the NIRS to be from 100%/0% (equivalent to raw data), to below 50/50 (Figure 1B). We can see as Hb/Mb is decreasing, (or the Mb contribution is increasing), apparent Hb saturation was lowered. Simulation suggests that below 50/50 of Hb/Mb estimation is not possible since the Hb at 4 minutes hit the lowest values below zero. On the other hand, Mb saturation was shifting down crossing the P_{50} values of 3.2 at about 4 minutes.

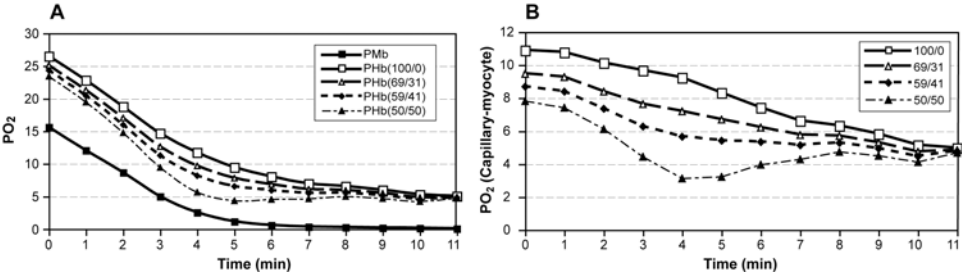

Figure 2. A; time course of PO_2, in myocyte (PMb, closed rectangle), and in the capillary (PHb) from the simulating various Hb/Mb (dotted lines) in human cuff ischemia. B; time course of PO_2 gradients between capillaries and myocytes.

We plotted the same data against PO_2 as Figure 1 (Figure 2A). The actual PO_2 depends on the Hb/Mb contribution on the NIRS. The possible actual capillary PO_2 (shown as PHb) was lowered as the contribution of Mb in the NIRS increased. The PO_2 gradients between capillary and myocytes is plotted in Figure 2B, which shows smaller PO_2 gradients between the capillaries and myocytes, if more Mb contributes in the NIRS during the time course, with possible f_{Mb} between 0% and 50%. In case of Hb/Mb 100/0, O_2 gradient starts from more than 10 torr at normal level, and ends about 5 torr at anoxia level. In the case of Hb/Mb contribution 50/50 in NIRS, the O_2 gradient was smaller (8 torr) at normoxia and decreased to 5 torr at anoxia, less different between the normoxia and hypoxia when the largest estimated Mb case was considered.

3.2. Canine Hypoxia Study with Sub-maximum Workload and Simulation

Preliminary dog studies showed that in order to obtain a full course of myoglobin desaturation in a relatively short time (within minutes), dog Gastrocnemius muscles required high submaximal workloads as well as limited supply of oxygen. In either one of these conditions, dog Gastrocnemius muscle did not show more than 30% desaturation. The FiO_2 at which half of the NIR and NMR signal becomes deoxygenated were 10 and 2.4%, respectively. These data suggested a much greater oxygen capacity in the dog than in the human skeletal muscles.

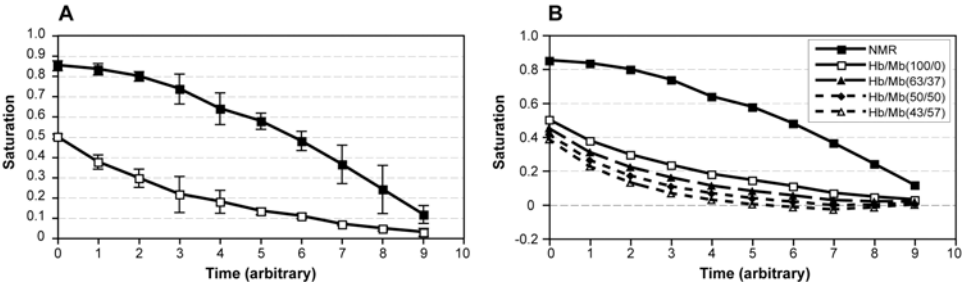

Figure 3. Saturation of Mb (NMR) and Mb+Hb (NIRS) were monitored during hypoxia to anoxia at submaximal workload (A). Simulated possible Hb/Mb and subsequently obtained Hb saturations were plotted as dotted lines (B).

In this study, in order to obtain whole spectral of desaturation in a relatively short time course, after a normal resting at 0 time, dogs were given low FiO_2 followed by anoxia while submaximal workloads were continued. Substantial Mb desaturation was seen when hypoxia was given at the submaximal stimulation (Figure 3A). In this figure, a relatively rapid phase of NIRS desaturation, at the beginning of the first 3 min was followed by later desaturation of NMR from 3 min to the end, similar to the human cuff study. However, NIRS desaturation was rather continuous in the dog study. We simulated possible Hb/Mb contribution in the NIR signals from Hb/Mb 100% (NIR signal is all due to Hb), to 43/57 (Mb is greater than Hb), and shown in Figure 3B. Note that in the case of 43/57 Hb/Mb, Hb saturation at a later time fell down below zero, which suggested the values were not adequately estimated and that the possible Hb/Mb contribution in the NIRS should be higher than 43/57. With increasing Mb contribution in the NIRS, the Hb desaturation curve was lower and steeper in the first 3 minutes. The simulation concluded that the maximum Mb contribution is near 50/% in the dog Gastrocnemius muscle, similar to the human muscle.

The PO_2 profiles from Mb and Hb saturation were plotted as myocyte and capillary PO_2, against time in the stimulated dog muscles (Figure 4A). The simulated Hb/Mb ranged from 100% to 43/57 are indicated in the dotted lines. We plotted PO_2 gradients between the capillary (PHb) and the myocytes (Pmyo) which were calculated from Hb and Mb saturation respectively, against time (Figure 4B). The possible oxygen gradients between capillary and muscle cells were larger with higher Hb contribution (less Mb contribution), ranging from 5 to 8.5 at rest and 4 and 9.5 torr at the highest (4-6 min) respectively in the dog muscles with hypoxia during submaximal workload.

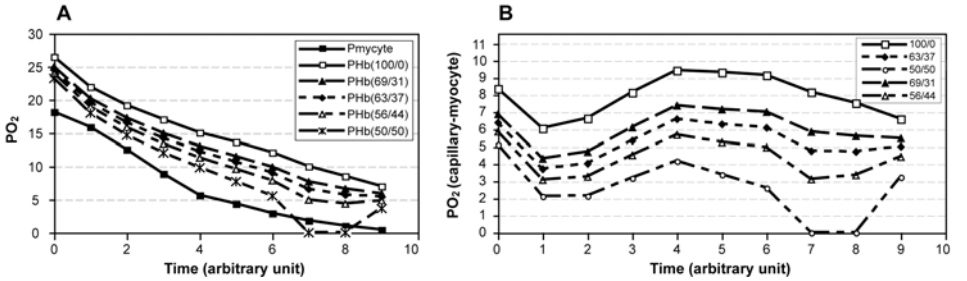

Figure 4. The PO_2 from Mb (Pmyo) and Hb saturation (PHb) were plotted against time in the stimulated dog muscles (A). The PO_2 values obtained from the simulated Hb/Mb ranged from 100% to 50/50 were shown as dotted lines. The same data are plotted as PO_2 gradients between capillaries and myocytes (B).

There appeared to be rather complicated trends of oxygen gradient with time, but the same trends were present in all cases of Hb/Mb. The trends consist of three different profiles of the oxygen gradient, even though both Mb and Hb curves against time were smoothly displayed in Figure 3. Here, the first profile, at time 0 and 1, shows that there is a reduction of oxygen gradient when dog muscle was under high workloads with mild hypoxia from the resting muscles. The second profile of oxygen gradients is that of increasing under an increasing amount of hypoxia during submaximal workload (from time 1 to time 4). And thirdly, when the oxygen gradient was already high at a high workload, it remained constant until oxygen was depleted and work no longer was achieved (after time 4 to end).

4. DISCUSSION

This study has demonstrated for the first time the possible oxygen gradients between capillaries and myocytes occurring in the human and dog muscles quantitatively, *in vivo*. The ^{1}H-NMR is used as a gold standard in providing an estimation of myocyte PO_2 and possible Hb/Mb contribution. Along with this knowledge, we can also estimate possible capillary PO_2 simultaneously using NIRS measured in the magnet.

The data showed that in the resting human arm muscle, limiting oxygen supply caused a linear reduction of O_2 gradient between capillary and myocyte. In addition, the greater the Mb contribution, the lesser the oxygen gradient. On the other hand, submaximally stimulated dog muscles with limited O_2 supply showed three O_2 muscle gradient profiles. Between resting to the submaximal working muscle, the O_2 gradient actually decreased. This was against our prediction, perhaps it occurs due to an excellent ability of increasing oxygen supply. The second profile showed an increased O_2 gradient during the submaximal workload, with more severe hypoxia at 3-6 arbitrary times, and it can be explained by limiting further oxygen supply. The last profile of the O_2 gradient was persistently constant in spite of more fetal hypoxia at the arbitrary time of 6 to 9 minutes. While oxygen supply was at risk, the work output was declining at the end of the time course.

With regard to the dog and human muscle responses to Mb and Hb desaturation, we have found that first, in order to desaturate 50% of the Mb, dog muscle needed to be in a more extreme condition (i.e. sub-maximal workload with hypoxia) while human muscle did not require as much (i.e. light exercise or cuff ischemia). This finding suggests a greater O_2 storing capacity in the dog muscle, which is also in agreement with the results

from Mb/Hb quantification[9]. Secondly, we have found that both dog and human muscles have equal Hb/Mb contribution in the NIR suggesting that Hb and Mb work hand in hand in delivering oxygen from the capillary to the mitochondria. In summary, simulation can help identify the possible Hb saturation in the capillary and with in vivo ^{1}H-NMR Mb measurement combined, we can better understand the dynamics of oxygen gradients between capillary and myocyte.

5. ACKNOWLEDGEMENT

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