

OXYGEN CONSUMPTION OF HUMAN SKELETAL MUSCLE BY NEAR INFRARED SPECTROSCOPY DURING TOURNIQUET-INDUCED ISCHEMIA IN MAXIMAL VOLUNTARY CONTRACTION

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INTRODUCTION

Among tissues, skeletal muscle shows the highest variability in energy turnover from the resting state through to maximal activity so that muscle is particularly suitable for investigation of metabolic regulation and oxygen consumption (VO_2) (Wittenberg and Wittenberg, 1989). Biochemical assessment of energetic turnover can be obtained "in vitro" by muscle biopsy (Hultman et al., 1990) and "in vivo" by ³¹P-NMR spectroscopy (Chance et al., 1986). Different experimental animal models have been employed to evaluate the relationship existing between the phosphorylation rate of energetic substrates and VO_2 . If VO_2 is not limited by O_2 availability, all models show a correlation between VO_2 and the phosphorylation state of the adenine nucleotides and/or creatinine (Mahler, 1985). Changes in respiratory chain enzyme redox states, independent of phosphorylation state, play a very minor role in regulating VO_2 in red muscle (Connett and Honig, 1989).

A non invasive measurement of muscle oxygenation can be obtained by fiber optic near infrared (700-1100 nm) spectroscopy (NIRS). NIRS has been developed experimentally and clinically to non-invasively monitor brain and muscle hemoglobin/myoglobin (Hb/Mb) oxygenation and cytochrome a-a₃ redox state (Tamura et al., 1989). Recently NIRS has been employed to measure the rate of muscular oxygen utilization on patients with mitochondrial myopathy (Sobolewski et al., 1990) and heart failure (Wilson et al., 1989). These studies provide only a qualitative analysis of Hb/Mb oxygen saturation. The evaluation of saturation changes has been conventionally made considering full saturation during 100% O_2 breathing and complete desaturation after 10 min ischemia (Hampson and Piantadosi, 1988). New techniques of time and frequency-resolved spectroscopy allow the measurement of the distribution of optical pathlengths in order to quantify the absorption changes of NIRS in tissues (Chance, 1991).

The aim of the present study was to evaluate the saturation

changes and VO_2 of human skeletal muscle at rest and subjected to maximal increase of energetic request. Muscle VO_2 can be measured by inducing an abrupt flow limitation and evaluating the Hb/Mb desaturation rate (Cheatle et al., 1990). Combining spectral information obtained by a fast scanning spectrometer with pathlength data measured with time resolved spectroscopy it is possible to quantify oxy and deoxy Hb/Mb concentration changes in the monitored area.

MATERIALS AND METHODS

Six healthy subjects were recruited from the laboratory. Informed consent was obtained from each subject. Spectral measurements were made using a fast scanning spectrophotometer (400-1100 nm) (mod. 6500, NIRSystems, Silver Spring, MD). The procedure for spectral analysis was described recently (Ferrari et al., 1989). Measurements were performed on the proximal forearm brachio-radial muscle. Two optic fibers (200 cm long and 0.5 cm active diameter) were applied 3-3.5 cm apart with a black rubber support so that a stable fiber geometry was achieved. NIRSystems software was utilized to automatically collect a scan every 5 sec. Each subject was submitted to two consecutive experiments.

After a stabilization period of 10 min, an abrupt flow interruption was achieved by a pneumatic cuff loosely wrapped around the arm. Arterial occlusion was obtained by inflating the cuff to a pressure of 240-260 mmHg. In the first protocol (A) two isometric maximal voluntary contractions (MVC) of 15 sec duration were executed 15 and 60 sec respectively after the beginning of ischemia. The cuff was released 145 sec after the occlusion started. In the second protocol (B) the cuff occlusion was maintained for 7 min in a resting muscle. The cuff was then released and a 3 min recovery phase followed.

Spectra were analyzed according to a modified Lambert-Beer law in order to obtain quantification of Hb/Mb changes during the experimental procedures. Difference spectra (ΔA) of the muscle tissue were calculated relative to the pre-ischemic period. These were converted into muscle absorption coefficient ($\Delta\mu_a$) using $\Delta\mu_a = \Delta A / (Bd)$ where d was the physical separation of the optodes on skin surface and B was 3.59, a factor which took into account the effective optical pathlength in muscle tissue (van der Zee et al., 1991). Changes in muscle absorption coefficient were assumed to result only from changes in the concentration of oxy-Hb/Mb and deoxy-Hb/Mb. The results were expressed as micromoles per liter of tissue ($\mu M/L$).

No difference in the absorption spectra of Hb and Mb in the near infrared region have been reported "in vitro" (Sassaroli and Rousseau, 1987). In this paper $[Hb]$ and $[HbO_2]$ represent the combined concentrations of deoxy-Hb/Mb and oxy-Hb/Mb respectively.

The tissue absorption coefficient spectra ($\Delta\mu_a$) were split into $\Delta[Hb]$ and $\Delta[HbO_2]$ using multilinear regression analysis (Cope et al., 1988) of the Hb and HbO_2 spectra (Wray et al., 1988). This statistical analysis led to values for the standard error of the $\Delta[Hb]$ and $\Delta[HbO_2]$ regression coefficients through the variance-covariance matrix and the sum of the squares of the residual errors at each wavelength. The regression analysis was performed over the wavelength region 750 to 900 nm with data points at 2 nm intervals.

O_2 was measured by calculating the rate of change of the conversion of oxy to deoxyhemoglobin in the isolated muscle $\times 0.5 d \{ \Delta[HbO_2] - \Delta[Hb] \} / dt$ and taking into account the molecular ratio between haemoglobin and oxygen. In protocol A O_2 was calculated during MVC, and in protocol B for the first 260 sec of ischemia when the desaturation process was linear. It was assumed that in that period changes in saturation were mainly due to haemoglobin. Note that the sum of $[Hb]$ and $[HbO_2]$ should represent changes in hemoglobin content as Mb content should not change during these experiments.

RESULTS

Figure 1 (left panel) shows a typical set of results of protocol A. After an initial ischemic period at 15 sec the addition of MVC provoked a faster desaturation rate that slowed when the MVC was interrupted. The second MVC, performed after 30 sec, did not give rise to any further desaturation and a plateau was maintained until 95 sec.

Cuff release was accompanied by a rapid recovery of hemoglobin content and a slower recovery of saturation. The

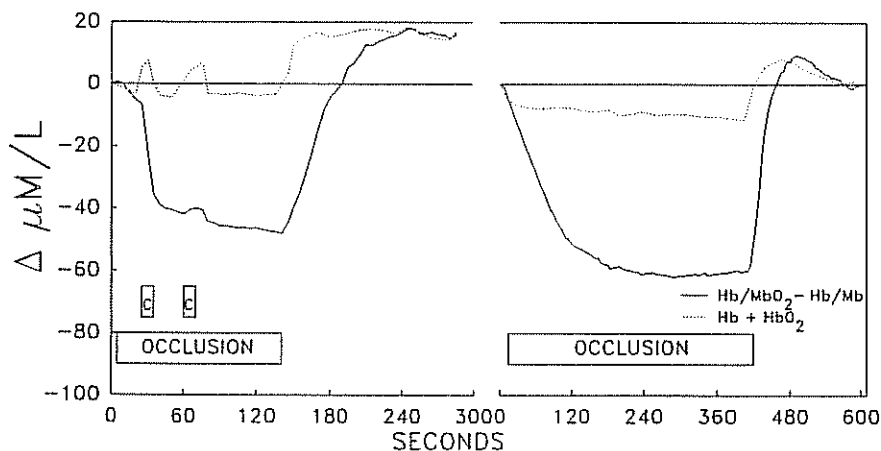


Fig. 1. Typical desaturation pattern during ischemia with (left panel) and without MVC (C)(right panel). The first MVC provoked a faster desaturation rate than that occurring in the occlusion without MVC.

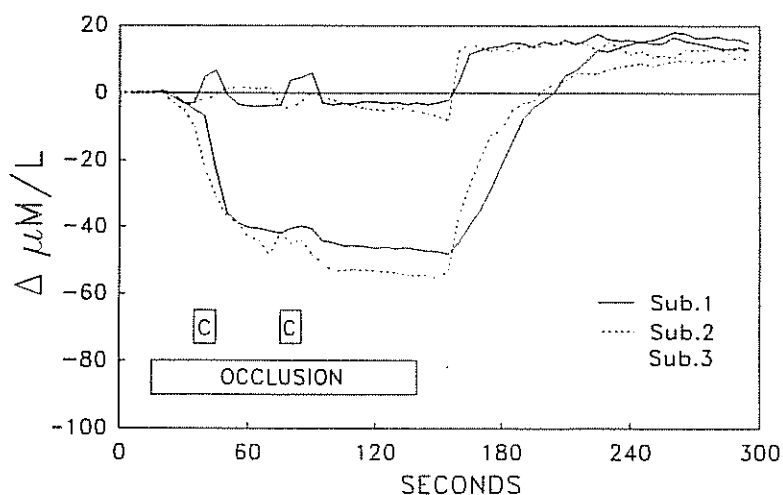


Fig. 2. Effects of MVC during ischemia on 3 different subjects. Upper tracing: $(Hb+HbO_2)$; lower tracing: $(Hb/MbO_2 - Hb/Mb)$.

occlusion without MVC provoked a slower desaturation rate but surprisingly the total desaturation was larger than that observed in the first protocol (Figure 1, right panel).

This effect is clearly shown in Figure 2 which displays protocol A performed on the subject of Figure 1 and two other volunteers. The mean \pm standard error of VO_2 for the 6 volunteers during procedure A and B are reported in Figure 3 (upper and lower panel respectively).

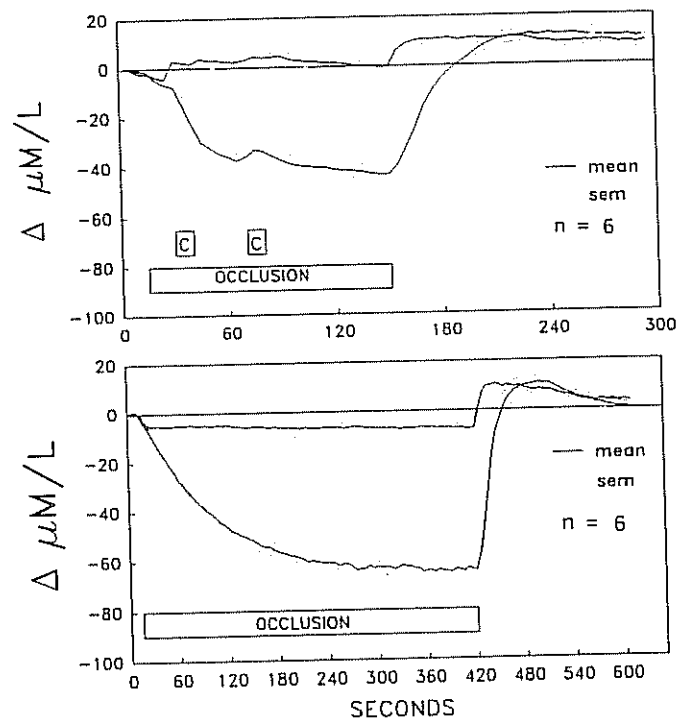


Fig. 3. Means \pm sem for 6 volunteers during procedures A and B (see text) are reported on upper and lower panel respectively. Upper tracing: (Hb+HbO₂); lower tracing: (Hb/MbO₂-Hb/Mb).

Table 1. Forearm muscle oxygen consumption during a constant tourniquet compression in resting condition and with MVC (means \pm sem; n=6).

N _o	Resting condition		MVC	
	VO ₂ $\mu\text{M}/\text{min}/$ 100gT	HbO ₂ -Hb $\mu\text{M}/\text{L}$	VO ₂ $\mu\text{M}/\text{min}/$ 100gT	HbO ₂ -Hb $\mu\text{M}/\text{L}$
1	3.07	61.05	19.94	45.27
2	4.15	60.71	23.55	42.45
3	4.15	60.61	12.36	41.00
4	3.34	68.08	21.11	41.87
5	6.77	37.89	18.14	35.89
6	7.58	66.76	11.91	32.75
Mean \pm sem	4.96 0.76	65.04 1.91	17.06 1.88	42.86 2.54

During constant tourniquet pressure in resting state muscle VO_2 was $4.96 \pm 0.76 \mu\text{M}/100\text{g}/\text{min}$ (mean \pm sem) (Table 1). In the six subjects in which the MVC was performed, oxygen consumption increased by $344 \pm 38\%$ of the resting value during the first MVC. Conversely no change of VO_2 was found during the second MVC. Maximum deoxygenation in the working and resting conditions are reported on the same table.

DISCUSSION

In resting skeletal muscle the heterogeneity in the Hb content and capillary flow involves a non uniform O_2 and substrate distribution that could limit the oxidative metabolism of a large number of cells (Duling and Damon, 1987). Consequently a limitation in muscular oxygen uptake would result despite an organ non-limiting availability of O_2 and substrates.

NIRS reflects the equilibrium between oxygen supply and consumption. In this study VO_2 evaluation was performed during the first 60 sec of the experimental procedures when Mb remained stable i.e. almost completely oxygenated due to its "in vivo" p_{50} of 5 mmHg (Gayeski and Honig, 1991). In addition Mb concentration is only 25% of total muscle Hb plus Mb concentration (Wang et al., 1990). The source of O_2 , either HbO_2 or MbO_2 , does not effect the VO_2 calculation, however dissolved oxygen in the cells will introduce a systemic underreading in VO_2 of up to 10%.

Our finding of a mean VO_2 of $4.96 \mu\text{M}/100\text{g}/\text{min}$ (range 3.07-7.58) in skeletal muscle at rest is lower than that reported in animal studies on leg utilizing flow data and the difference between the arterial and venous oxygen content (Duran and Renkin, 1974). The results obtained by Cheatle et al. (1990) on human leg by NIRS show values ranging from 4.46 to 24.55 $\mu\text{M}/100\text{g}/\text{min}$. The difference could be explained by the different muscle groups studied. The broad variability of data found here was related to differences in VO_2 among subjects. Multiple measurements performed on the same subject showed smaller deviations.

Studies on muscle energy regulation report two different types of respiratory control at rest and at work. At rest, muscle respiration undergoes extrinsic control by O_2 and substrate availability to cells (Chinet and Mejsnar, 1989). The observation of a relatively stable deoxygenation level 4 min after the occlusion can be explained by a rapid activation of anaerobic glycolysis leading to negligible oxygen consumption. In working muscle, phosphate energetics and/or calcium modulated enzymes activities within the mitochondria may account for VO_2 control, while the respiratory chain enzymes play a very small role (Connett and Honig, 1989) and the availability of O_2 (Gayeski et al., 1985) and substrates are not limiting factors (Wolfe et al., 1987). This could explain our finding of a significantly smaller degree of maximal deoxygenation in working muscle with respect to the resting state.

The presence of respiratory suppression with O_2 supply to the cells is also supported by the occurrence that in working skeletal muscle O_2 gradients from sarcolemma to cell interior are very shallow because of myoglobin-facilitated O_2 diffusion (Gayeski and Honig, 1986).

In conclusion:

- 1) fast scanning spectroscopy and path length data allow the measurement of oxygen consumption of skeletal muscle at rest and working conditions,
- 2) O_2 extraction is inhibited despite the presence of a significant concentration of O_2 both in working and resting muscle,
- 3) in ischemic muscle the maximal deoxygenation in the working condition is less than that observed at rest,
- 4) in working muscle VO_2 is not due to the reduced O_2 availability.

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