

Simultaneous Measurement of Tissue pCh and Perfusion During Muscular Contraction of the Latissimus Dorsi

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Abstract

The latissimus dorsi muscle (LDM) receives its primary blood supply from a single nutrient artery located at its proximal insertion and it has not previously been established whether this affects the regional perfusion and nutrition of the muscle. A microelectrode technique was used to measure local oxygenation and perfusion simultaneously at multiple sites in the rabbit LDM at rest, during and after repeated muscular contraction induced by electrical stimulation with a fatigue-testing protocol. In the resting state there were no significant differences in perfusion or oxygenation in different regions of the LDM. However, the proximal perfusion was greater than distal perfusion during muscular contraction (increase from baseline $173 \pm 9\%$ and $137 \pm 5\%$) and during the first 20 minutes of recovery. Tissue oxygenation was maintained at constant levels in the proximal region throughout contraction and recovery whereas there was a significant decrease in distal pO₂ of 10.2 ± 5.5 mmHg during contraction and of 5.4 ± 4.2 mmHg during the first 20 minutes of recovery. The evidence suggests that distal areas of the LDM cannot maintain sufficient perfusion during contraction to prevent a fall in tissue pO₂. This has important implications on the clinical applications of the LDM as a pedicled muscle flap for cardiac assistance.

Key words: microelectrodes, nitrous oxide clearance technique, electrical stimulation, micro-circulation, skeletal muscle metabolism.

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The latissimus dorsi muscle (LDM) is a broad, flat based muscle which receives its major blood supply from the thoracodorsal artery located at its very proximal pole [7]. This anatomy makes the muscle ideally suited for use as a pedicled flap in the fields of plastic surgery and, more recently, in cardiac surgery where it is used as a muscle wrap to support a failing heart. However, the unidirectional nature of the blood supply in such a large muscle raises the question whether perfusion is uniform across the muscle bed. Any such heterogeneity may influence the viability of the muscle in its clinical applications or in its ability to adapt to conditioning. Conditioning is the process by which fast-twitch fibres are transformed into slow-twitch fatigue resistant muscle through an appropriate regime of electrical stimulation - a process which is essential to maintaining cardiac assistance and which involves major changes in capillary density and muscle metabolism [26]. In studying the feasibility of such applications we feel it is important to investigate the relationship between perfusion and nutrition in the LDM both under resting conditions and during electrical stimulation.

Tissue oxygenation and perfusion were measured volumetrically using a further development of methodol-

ogy we have employed in previous work on connective tissue [20], skeletal muscle [9, 10] and the myocardium [6]. In the previous studies we used the inert gas clearance technique of measuring perfusion, arguing that in poorly perfused tissues or during ischaemia, diffusible solutes provide a clearer picture of nutrient exchange than do particulate tracers. They provide information of transport through the tissue matrix and all components of the micro-circulation and our work has suggested that this technique uncovers components of perfusion which are not detected by microspheres and that these pathways become increasingly important in areas of regional ischaemia [3, 6, 34].

In our previous studies we, like many other previous workers employed hydrogen as the tracer gas [5, 25]. However, the use of hydrogen in the open laboratory can be hazardous and in the present study we use nitrous oxide as the perfusion tracer. In a recent paper we have demonstrated that the kinetics of its wash-in and clearance are indistinguishable from hydrogen [25]. Nitrous oxide is detected by reduction on a silver electrode and the advantage of this technique is that by voltage-stepping the same electrode can be used to measure both perfusion and oxygenation, offering theoretical and practical advantages

over previous studies in which we used two juxtaposed electrodes [6, 9]. The electrochemical aspects of this method are detailed elsewhere [5,20],

This study applies these microelectrode techniques to the LDM and establishes the variation that exists in the resting perfusion and tissue oxygenation in different regions of the muscle, firstly in terms of both spatial and temporal heterogeneity and secondly with respect to the variability seen between individuals. Electrical stimulation was then applied to the muscle to generate repeated contractions to investigate the regional changes seen during exhaustive exercise and recovery.

Methods

Electrode measurements

The microelectrodes were purpose built and consist of a 125 μ m diameter insulated silver wire embedded in epoxy resin within a 23G butterfly needle (fig 1). The wire was cut on a clean surface using a scalpel blade and the polyester insulation coating removed from the proximal 5 mm. This was wound around the exposed core of a length of screened cable and soldered with low-melting point tin. The wire was then threaded through a 25 mm long, hollow barrel of a 23 G needle and the proximal end was protected with a 10mm length of nylon tubing. This assembly was then potted with epoxy resin (Ciba Geigy). When set, the electrode tip was cut obliquely to give it a bevelled tip and initially ground on a lathe using progressively finer grades of emery paper until the tip appeared smooth under a microscope. Polishing was completed using five grades of diamond paste and finally, a slurry of alumina paste on a

lathe followed by hand polishing on a felt pad. The electrodes were washed in ethanol and deionised water after each stage and examined under the microscope until no surface imperfections could be seen, creating a mirror-like silver microdisc electrode. At completion of this process the electrodes were cleaned in an ultrasound bath and stored in deionised water.

Previous work has shown that this electrode design causes damage only to the cells in immediate contact with the surface and does not otherwise disrupt the tissue architecture [10, 19,31]. The size of the electrodes is such that the sampling area is large enough (equivalent to a hemisphere of tissue 750 μ m in diameter [32]) to extend well beyond the neighbouring cells and include a representative area of tissue.

Surgical procedure

Ten male New Zealand White rabbits weighing approximately 2.5 kg were anaesthetised using intra-muscular Fentanyl-Fluanisone (Fentanyl citrate 0.315 mg/ml, Fluanisone 10 mg/ml. Hypnorm, Janssen Pharmaceutica, given i.m. 0.2 ml/kg) and intra-venous Midazolam 1 mg/ml. Anaesthesia was maintained with a continuous infusion of Midazolam and further im boluses of Fentanyl-Fluanisone 0.1 mg/kg hourly. A tracheostomy was performed and the animals ventilated using a standard Harvard rodent ventilator. Blood-pressure was monitored via a carotid artery line which also allowed access for blood-gas sampling. Ventilation was controlled to maintain pH 7.36-7.44 and pCO₂ at 3.5-5.0 kPa. Body temperature was maintained between 36.5-38°C using a heating blanket and saline was given at 5 ml/kg/hr intravenously to replace insensible loss.

A longitudinal incision was made immediately below the point of the scapula to expose the latissimus dorsi muscle and two microelectrodes were placed intra-muscularly one distally, one proximally. The epimysium was removed with a scalpel blade and the microelectrode introduced, along the plane of the muscle fibres so that it lay within the parenchyma of the muscle 1-2 mm below the surface. The exposed muscle was coated with a warm saline-soaked swab to prevent it desiccating and to reduce data contamination by diffusion to and from the surroundings.

A calomel electrode (Russell) acting as counter and reference was placed subcutaneously in the groin and connected to a potentiostat (Energy Microsystems, Oxford, UK) interfaced by an A/D Converter (Strawberry Tree Graphics, USA) to a personal computer. Voltage cycling and data acquisition were performed using Workbench software (Strawberry Tree Graphics). Previous experiments [5,25] demonstrated that the optimum operating protocol was to hold the voltage at approximately -0.6 V to measure oxygen followed by switching to approximately -1.2 V to measure the reduction current for nitrous oxide. The exact operating voltage for each electrode were determined from the plateau of the current-voltage curve measured in calibration solutions of PBS immediately

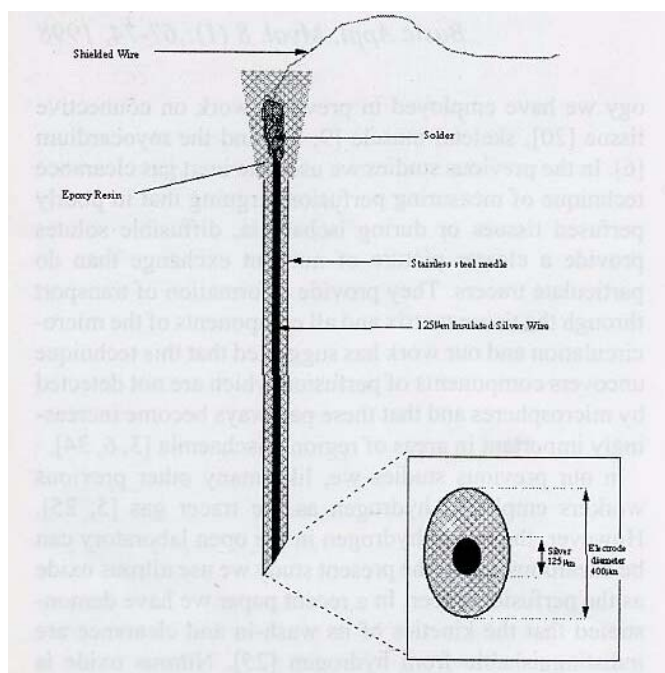


Figure 1. Components of the intra-muscular micro electrode. Inset shows the appearance of the tip seen end-on to view the working surface.

before use and were checked immediately the electrodes were removed at the completion of the experiment. Current-voltage curves were also plotted with the electrode in the muscle to confirm that the chosen operating voltages were appropriate *in vivo*. The electrodes were inserted into the resting muscle and allowed to settle for 30 minutes before any measurements were made. The electrodes provided stable currents for both analytes and the pO_i did not alter significantly from that determined by a gold electrode [25].

Tissue perfusion was measured by administering 20% nitrous oxide via the tracheostomy tube until the current recorded by the electrode reached a plateau. Ventilation was then returned to room air and the clearance curve was obtained. Perfusion was calculated from the slope of the log[nitrous oxide current] v. time plot as described previously [25, 33].

The muscles were stimulated using a programmable pulse generator (Master-8-cp, A.M.P.T., Israel) via two epimyseal electrodes placed either side of the thoraco-dorsal nerve. The muscles were stimulated at 50 Hz for 200 ms with each pulse duration being 14 μ s. This produces a sustained tetanic contraction in accordance with the protocol of Burke et al, for fatigue testing [2].

The muscle was divided into fifteen regions for measurement as shown in fig. 2. The most proximal point was the insertion into the humerus and the distal point was the aponeurotic origin. The length of the muscle was then divided by four equidistant lines to give five regions along the proximal-distal axis numbered 1 to 5. Transversely the muscle was divided into three equal regions (medial, lateral and central) labelled 'm', 'l' and 'c' as shown in fig. 2. A series of preliminary experiments were carried out on resting muscle to establish the baseline values and heterogeneity for regional flow and oxygenation within the muscle. Measurements were obtained longitudinally (i.e. from proximal to distal) and transversely (i.e. from medial to lateral) within the muscle. Subsequent experiments focused on the regions that differed most such that the number of working electrodes could be kept to a minimum.

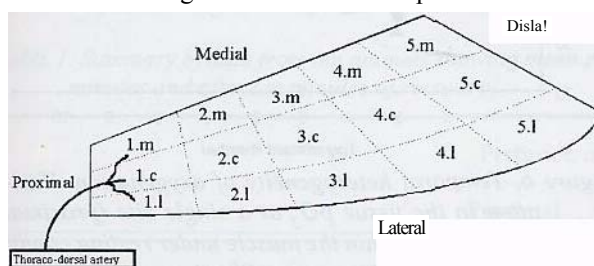


Figure 2. Diagrammatic representation of the latissimus dorsi muscle of the rabbit showing the point of entry of the thoraco-dorsal vessels. The muscle was divided into fifteen regions as shown by measuring the dimensions of the muscle edges and dividing into five longitudinally and three transversely. Regions were numbered 1 (proximal) through 5 (distal) and suffixed with m (medial), c (central) or l (lateral).

Perfusion and oxygenation measurements were taken at rest, during muscle stimulation and during the period of recovery for a further 30 minutes. All animals received humane care in accordance with the guidelines published by the National Society for Medical Research (Principles of Laboratory Animal Care) and by the National Institutes of Health (Guide for Care and Use of Laboratory Animals). The project was licensed and performed under the supervision of H.M. Inspector from the Home Office.

Results

A typical nitrous oxide wash-in and wash-out current is shown in Fig. 3 together with the log plot of the clearance curve. The inhalation of 20% N₂O did not cause any significant reduction in blood-pressure; the systolic BP in fifty inhalations of N₂O was 129 ± 6.8 mmHg compared to 126 ± 8.2 mmHg breathing room air.

Resting muscle

A considerable heterogeneity was seen in oxygenation and perfusion. This was examined both between individuals and within the same individual in terms of both spatial and temporal changes.

A preliminary study of spatial heterogeneity was carried out in a group of five animals, in which three measurements were made across the transverse axis of the muscle (medial, lateral, central) compared with three points along the longitudinal axis of the muscle (at points 1, 3 and 5 as shown in fig. 2). This gave a total of 15 sets of data in each axis and the variation within each set of values was analysed. The mean variation in perfusion in the transverse axis was $1.77 \text{ ml.min}^{-1} \text{ } 100\text{g}^{-1}$ and longitudinally was $6.84 \text{ ml.min}^{-1} \text{ } 100\text{g}^{-1}$. Values for tissue oxygenation showed less variation, but again the differences were more marked in a longitudinal pattern than transversely across the muscle (mean standard deviation 2.8 and 5.0 mmHg respectively). The variation from proximal-distal was significantly different from the transverse variation for both perfusion ($p < 0.01$) and oxygenation ($p < 0.05$). In the light

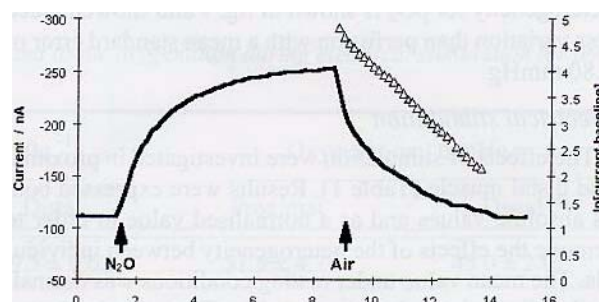


Figure 3. Curve obtained during wash-in and wash-out of inhaled 20% nitrous oxide. The first arrow indicates when the animal was ventilated with N₂O and the second arrow indicates when ventilation was returned to room air. The log-plot of the washout phase (A) is plotted against the secondary axis.

Regional muscle pO₂ and perfusion

of these observations we made a closer examination of the proximal-distal variation.

Perfusion

The mean values for muscle perfusion were 20.4 ± 0.6 ml.min⁻¹.100g⁻¹ for the proximal muscle and 19.5 ± 0.6 ml.min⁻¹.100g⁻¹ for the distal muscle (see Table 1). This difference was not significant (0.9 ml.min⁻¹.100g⁻¹ 95%CI -3.3 to 5.16, $t = 0.559$ $p = 0.58$). The distribution in resting perfusion is shown in figs. 4 and 5. The "spatial heterogeneity" in perfusion across the muscle bed of each animal ($n = 10$) is shown in fig. 4. The figures are all taken at rest and recorded at five different sites within the muscle (1.c - 5.c, fig. 2). Each point represents the mean of three consecutive values taken over 30 minutes. The mean perfusion figures decrease from proximal to distal with a Spearman Rank correlation of 0.88. The ranges of values obtained at each point varied from 9.2 to 20.6 ml.min⁻¹.100g⁻¹. There was no significant difference between the mean values at each point.

Fig. 5 shows the "temporal heterogeneity" that was measured at the same point within the muscle of each animal ($n = 10$). Electrodes placed in the proximal muscle (point 1.c, fig. 2) recorded perfusion over a period of 90 minutes under constant conditions (ie. in resting muscle with constant temperature, blood-pressure and blood-gases). The mean standard error for resting perfusion within the same animal (intra-muscular variation) was 2.81 ml.min⁻¹.100g⁻¹. This was less than the inter-muscular variation derived from the data between animals which was 4.65 ml.min⁻¹.100g⁻¹ ($p < 0.05$).

Oxygenation

Mean values for tissue pO_i were 51.5 ± 16.3 mmHg for the proximal muscle and 45.0 ± 14.2 mmHg for the distal muscle, giving a mean difference of 6.5 mmHg (95% CI -3.1 to 16.1, $t = 1.53$, $p = 0.08$). Spatial variation in tissue pO_i is shown in fig. 6. As for perfusion, the inter-animal variation (standard error 4.5 mmHg) was greater than the variation seen within each animal (standard error 1.9 mmHg). Correlation between site and tissue pO_i was not as strong as for the perfusion values ($r = 0.72$). Temporal heterogeneity for pO₂ is shown in fig. 7 and showed much less variation than perfusion with a mean standard error of 1.80 mmHg.

Electrical stimulation

The effects of stimulation were investigated in proximal and distal muscle (Table 1). Results were expressed both as absolute values and as a normalised value in order to remove the effects of the heterogeneity between individuals. The mean value under resting conditions was normalised to unity in each case, and the effects of stimulation were expressed as a fractional change in oxygen current or perfusion.

Perfusion

A summary of the perfusion changes with stimulation and recovery is shown in fig. 8. Taking all the results (proximal and distal) together they demonstrate an in-

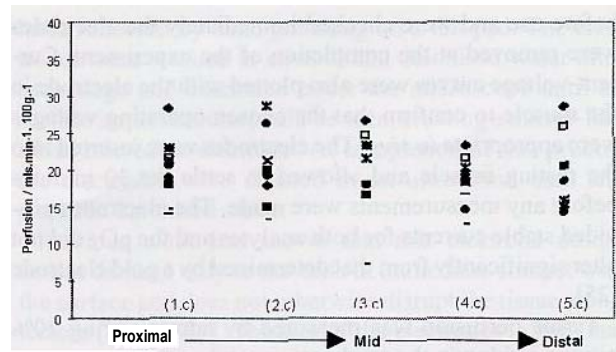


Figure 4. Spatial heterogeneity of perfusion. Perfusion at five sites within the latissimus dorsi muscle recorded in ten animals. Each value is the mean of three perfusion measurements taken over a 30 minute period. Figures in parentheses along the x-axis refer to muscle regions in Fig. 2.

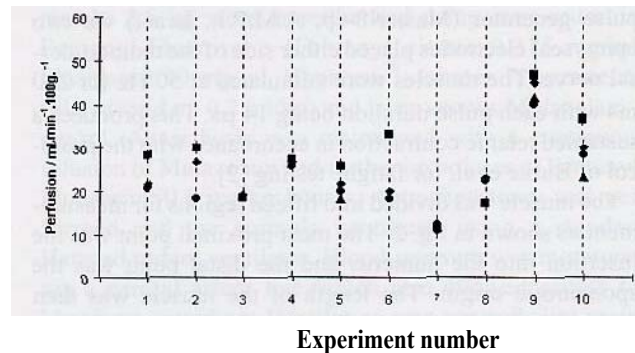


Figure 5. Temporal heterogeneity of perfusion. Distribution of perfusion data obtained every 15 minutes over a period of 90 minutes in ten subjects- All recordings were made in the proximal region of the muscle

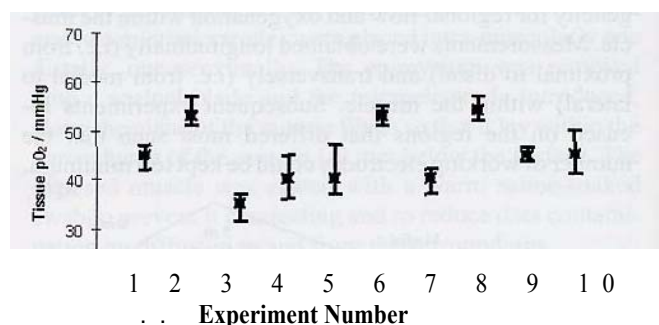


Figure 6. Temporal heterogeneity of oxygenation. Variation in the tissue pO₂ at a single site (proximal region, 1.c) within the muscle under resting conditions in ten subjects over a 90 minute period. Mean value and range of values shown.

crease in perfusion occurring during muscle activity by a mean of 11.0 ml.min⁻¹.100g⁻¹ or of 155% relative to the resting value in each case. The absolute increase was significantly greater in the proximal muscle than in the distal muscle (14.0 versus 8.0 ml.min⁻¹.100g⁻¹, $p <$

Regional muscle pO₂ and perfusion

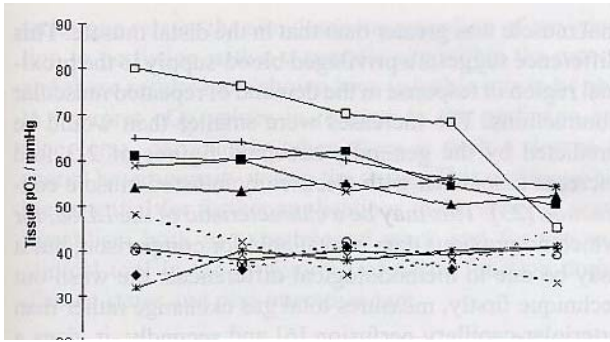


Figure 7. *Spatial heterogeneity of oxygenation. Variation in tissue pO₂ at five different points within the latissimus dorsi muscle in ten animals. Figures along the x-axis refer to muscle regions in Fig. 2.*

0.05). which was also reflected in the normalised change (173% versus 137%, $p < 0.05$).

During recovery the perfusion remained elevated compared to resting values for up to 30 minutes and proximal perfusion remained significantly higher ($p = 0.04$) than distal values for the first 20 minutes of recovery. Distal perfusion remained elevated above baseline for longer than the proximal region.

Oxygenation

A summary of the tissue pC*2 changes with stimulation and recovery is shown in fig. 8. The changes in oxygenation that occurred with stimulation fell into two distinct patterns. The proximal muscle showed no significant change in the tissue pO₂ during and after muscular activity. In individual cases the pO₂ actually increased during exercise but overall, the mean differences from resting values were not significant. The distal muscle showed a decrease in tissue pO₂ by a mean of 10.2 mmHg (24.5%) compared to resting values (see Fig. 9). The values for pO₂ that occurred with stimulation were significantly lower in the distal muscle ($p = 0.002$) and gradually returned to resting values during recovery. The pO₂ remained significantly lower in the distal region compared to proximal after both

Table 1. Summary of data from ten animals showing mean perfusion and tissue oxygenation during electrical stimulation for ten minutes and after ten minutes of recovery.

	Perfusion/ml.min. ⁻¹ 100g. ⁻¹		Oxygenation / mmHg	
	Proximal	Distal	Proximal	Distal
Resting Stimulation:	20.4 ± 0.55	19.5 ± 0.62	51.5 ± 4.78	45.0 ± 2.07
change from resting value normalised	14.0 ± 0.77 0.73 ± 0.09	8.0 ± 1.85 0.37 ± 0.15	7.9 ± 3.88 0.2 ± 0.11	-10.2 ± 3.80 -0.2 ± 0.08
Recovery: change from resting value normalised	17.33 ± 1.47 0.90 ± 0.20	8.49 ± 1.55 0.43 ± 0.13	1.2 ± 4.98 0.1 ± 0.11	-5.4 ± 4.24 -0.1 ± 0.10

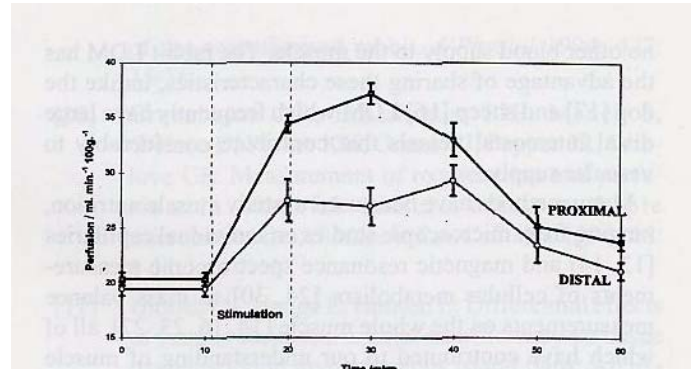


Figure 8. *Summary of changes in mean perfusion during electrical stimulation and recovery.*

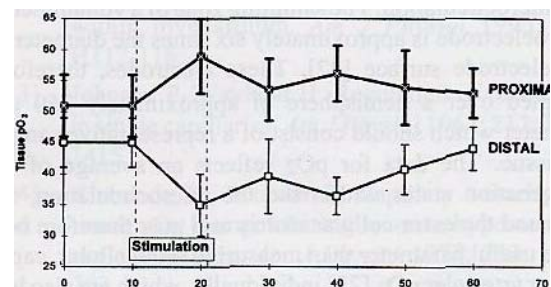


Figure 9. *Summary of changes in mean tissue pO₂ during electrical stimulation and recovery. Data is shown as mean ± standard error.*

ten ($p < 0.05$) and twenty ($p < 0.05$) minutes of recovery.

Discussion

The anatomy of the latissimus dorsi muscle has significant implications for its use in the fields of plastic, reconstructive and cardiac surgery. In the human, the blood-supply comes predominantly from the thoracic-dorsal axis, a branch of the subclavian artery that reaches the proximal body of the muscle as it winds around the scapula. Other than a few small intercostal perforators there is

no other blood supply to the muscle. The rabbit LDM has the advantage of sharing these characteristics, unlike the dog [17] and sheep [16] LDM which frequently have large distal intercostal vessels that contribute considerably to vascular supply.

Many methods have been used to study muscle nutrition, ranging from microscopic studies on individual capillaries [13, 14] and magnetic resonance spectroscopic measurements of cellular metabolism [24, 30] to mass balance measurements on the whole muscle [14, 16, 23, 27], all of which have contributed to our understanding of muscle metabolism. In the present study we sought to use microelectrodes to obtain representative values of perfusion and oxygenation over a volume of tissue comprising of cells, interstitium, arterial, venous and all components of the microcirculation. The sampling zone of a volammetric micro electrode is approximately six times the diameter of the electrode surface [32]. These electrodes, therefore, sampled over a hemisphere of approximately 750 μ m diameter which should consist of a representative sample of tissue. The data for pO₂ reflects an average of the oxygenation states within the the microcirculation, the cells and the extra-cellular matrix and may therefore be a more useful parameter than measuring in trace llular, capillary or arteriolar pO₂ [22] individually, which are also less practicable in terms of producing a tool for clinical measurement.

The mean pO₂ of 30-45 mmHg and perfusion of 15-20 ml.min⁻¹ 100g⁻¹ are in keeping with previous studies using a variety of techniques [9, 11, 21, 24, 28]. In the resting muscle we found a considerable heterogeneity of both perfusion and oxygenation, which supports what has been reported elsewhere [12,21]. Variation between individuals was greater than that seen within the same animal and this effect could be reduced by expressing values relative to their original resting value, each acting as their own baseline. The studies in resting muscle demonstrated that although there is a degree of heterogeneity from point to point within the muscle, the most significant feature was a variation along the proximal-distal axis. The tissue oxygenation showed less variation suggesting that perfusion is continually changing within the muscle bed in a manner which serves to maintain the tissue pO₂ at a constant value.

Previously, measurements have been performed in the tibialis anterior muscle [9, 10] which, unlike the LDM, does not have a polarised blood-supply. The range of values recorded were comparable for both muscles but in LDM there is a regional pattern of perfusion which can be related to its proximal arterial supply. Tibialis anterior has traditionally been used in the rabbit as a typical fast-twitch muscle, but the LDM is much more relevant to the clinical applications of reconstructive muscle flaps and conditioned muscle flaps for cardiac assistance.

Our measurements showed active hyperaemia and subsequent reactive hyperaemia during and after stimulation. The perfusion values rose throughout the muscle in response to muscular activity, but the increase in the proxi-

mal muscle was greater than that in the distal muscle. This difference suggests a privileged blood-supply to the proximal region in response to the demand of repeated muscular contractions. The increases were smaller than would be predicted by the generally accepted figures of 2-3 fold increase associated with electrically induced muscle contraction [23]. This may be a characteristic of the LDM, for which no previous data is available for comparison, but it may be due to methodological differences: the wash-out technique firstly, measures total gas exchange rather than arteriolar-capillary perfusion [6] and secondly, it gives a figure for the average perfusion over several minutes, transient changes in perfusion may be temporarily higher.

The regional changes in pO₂ have not been demonstrated before. In the well-perfused proximal muscle there is sufficient flow reserve to supply the increased metabolic demands of stimulation, indeed the tendency is almost to outstrip this demand in terms of oxygen supply and frequently the pO₂ rises slightly during hyperaemia. This is evidence to suggest that a fall in pC_b alone is not the only factor that autoregulates the active hyperaemic responses and supports the work of Duling [4] and Johnson [13] who have suggested that there are probably several metabolic and myogenic effectors exerting a combined effect, these include pCO₂ (and pH) aswell as possibly hyperkalaemia phosphates, adenosine, prostaglandins, cytokines and total osmolarity [23]. There is a different response in the distal muscle, where perfusion is less and although it has sufficient flow reserve to generate active and reactive hyperaemia, it is unable to keep up with the oxygen demand completely, resulting in a fall in the overall pO₂ during exercise and a subsequent longer period of reactive hyperaemia, suggesting that a greater metabolic debt had developed in the distal muscle compared to the proximal muscle. We hypothesize that this region is particularly at risk during electrical stimulation and that conditioning protocols that call for repetitive periods of such activity should take account of this heterogeneity.

The electrodes are robust and reusable and they cause minimal disturbance to the tissue [6,10,19, 31]. There was no evidence of surface poisoning of the electrodes during the time-course of these experiments, calibrations performed immediately before and after each experiment were unchanged. The validation of nitrous oxide as the marker for perfusion has overcome the expense, hazards and technical problems of using hydrogen. There was no evidence that 20% nitrous oxide causes cardio-vascular depression nor that the kinetics of clearance were any different to those of hydrogen. The advantage of the microelectrode technique described here is that perfusion is measured through the dynamics of the delivery of the inert gas to tissue itself which reflects transport by diffusion and convection through the interstitium and does not depend purely on the blood-flow. This theoretical advantage is accompanied by practical advantages over comparable techniques such as the use of panicle tracers [1, 15] which cannot be used in a clinical environment. This

technique relates the simultaneous recording of oxygenation to perfusion within a specific site within the muscle and these results have shown how valuable it is to be able to interpret pCb values in relation to the perfusion and vice-versa, particularly when there is such a degree of spatial heterogeneity within the tissue. This technique has the potential for further applications in the study of tissue metabolism both in experimental work and for use as a clinical tool in the monitoring of tissue function during disease states and peri-operative care.

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