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Continuous percutaneous measurement by laser-Doppler flowmetry of skeletal muscle microcirculation at varying levels of contraction force determined electromyographically

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Summary. Laser-Doppler flowmetry (LDF) and electromyography (EMG) were used simultaneously for measuring skeletal muscle blood perfusion in relation to static load and fatigue. Percutaneous single-fibre LDF and bipolar surface EMG of the trapezius muscle were performed continuously during a 10-min series of alternating periods of static contractions and rest, each of 1-min duration. The muscle was exposed to static load expressed as shoulder torque, by keeping the arms straight and elevated at 30, 60, 90 and 135°. On-line computer processing of the LDF and EMG signals made possible the interpretation of the relationship between the perfusion and the activity of the muscle. The LDF and root mean square (rms)-EMG were normalized by using the average value of the serial examinations of each individual as a reference value. Spectrum analyses of EMG showed the lowest variability for median frequency (MDF) in the frequency range 10–1000 Hz and mean power frequency (MPF) at 2–1000 Hz. The LDF power spectrum density during low (muscle rest) and high (high-force muscle contraction) perfusion indicated that disturbances were small when measurements were performed during sustained static contraction with as little movement as possible. Vasomotion, i.e. rhythmic variations in the blood flow, were present and showed a frequency of 5–6 cycles·min^{-1}. Application of a tourniquet to the upper arm caused an arrest of the microcirculation in the distally situated brachioradial muscle which was followed by a postischaemic hyperaemia upon removal of the torniquet. In ten healthy men, regression analyses showed positive correlation between rms-EMG and shoulder torque ($r = 0.77$), negative correlation between MPF and arm elevation angle ($r = -0.89$) indicating accumulated fatigue, and almost positive correlations between LDF and rms-EMG ($r = 0.65$), and between LDF and shoulder angle ($r = 0.67$) when the right trapezius muscle was examined.

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Key words: Laser-Doppler flowmetry – Skeletal muscle – Static muscle contractions – Electromyography – Spectral analyses – Muscle fatigue – Skeletal muscle perfusion

Introduction

Skeletal muscle blood flow has attracted much interest, particularly in relation to muscle fatigue. For studies of individual muscles, the Xenon-133 washout technique introduced by Lassen et al. (1964) has been the one most often used. It has been shown to give reliable results of qualitative changes under various physiological conditions, although it underestimates muscle blood flow quantitatively (see Cerretelli et al. 1984). For studies of the effects of muscle work of one extremity, thermodilution techniques have been used for measurements of blood flow and pressure, catheters having to be placed in the main artery and vein for blood sampling (see Andersen and Saltin 1985). None of these methods has offered the possibility of measuring continuously dynamic changes in local muscle blood flow in relation to current muscle work.

Laser-Doppler flowmetry (LDF) as introduced by Stern (1975) measures tissue blood perfusion as the Doppler-effect (Nilsson et al. 1980a, b). Measurements of microvascular blood flow in skeletal muscle were first reported by Öberg et al. (1979). The LDF blood flow data would seem to have shown a linear relationship to several other methods such as microspheres, hydrogen washout to direct measurements of volume flow as reviewed by Tyml et al. 1990. For flow rates above 10 ml·min^{-1}·100 g^{-1} tissue, LDF has seemed to underestimate flow to a certain extent.

Conventional laser-Doppler techniques used up to now have necessitated surgical exposure of the muscle for application of the probe, as practised in a previous combined biopsy study (Larsson et al. 1990). A similar study has been one in patients with neuromuscular diseases by Tahmoush et al. (1983). The further develop-

ment of fibre optics with the introduction of a single-fibre technique by Salerud and Öberg (1987) has increased the possibilities of studying deep tissue perfusion under various physiological conditions. This technique has been applied in the present study and shows the possibility of performing percutaneous flowmetry clinically. A preliminary report has been given by Larsson and Öberg (1990).

The object of this study was to measure continuously muscle microcirculation in relation to varying loads.

Methods

Subjects. Ten healthy men aged 23–57 years (mean 40 years) were examined. Their body mass was 63–90 kg (mean 76 kg) and body height 167–188 cm (mean 177 cm). All did light work with no static load involved. No one had complaints from the neck-shoulder region, which could have influenced the outcome of the study and none took medication, e.g. for hypertension or for any other disease. Two of the ten men were habitual smokers with moderate daily cigarette consumption. All agreed to volunteer. Consent to the study was given by the Research Ethics Committee at our University Hospital.

Exposure to static load. The right as well as the left trapezius muscles were simultaneously exposed to static load from the mass of the arms for periods of 1 min with 1 min of rest in between. The individual sat upright on a standard office chair with relaxed, hanging arms (rest position). On command he raised his arms straight and symmetrically in the scapular plane (approximately midway between abduction and flexion) to 30, 60, 90 and 135°, i.e. the load positions. The LDF and electromyogram (EMG) signals were recorded continuously (Fig. 1). Torque in the shoulder joint was calculated by standard biomechanical methods. The computations were based on the height and mass of the person as described in Chaffin and Andersson (1984) and the mass and centre of mass of the arm according to LeVeau (1977).

Procedure. The LDF was used for continuous measurements of the blood perfusion in the upper portion of the right trapezius, the deltoid and the brachioradial muscles at varying isometric tensions. For continuous recordings of the trapezius muscle, an optical single fibre with a diameter of 0.5 mm was percutaneously placed within the muscle halfway between the spinous process of the C7 vertebra and acromion (Fig. 1). To lead the optical fibre to the maximal depth for the recordings, a plastic cannula (Venflon 2 iv cannula, 1.0 mm OD, Viggo Co., Helsingborg, Sweden) had first been inserted into the muscle under local anaesthesia of the skin. Insertion was made 5–10 mm deep from the point where the subject noticed the somewhat painful passage of the cannula through the muscle fascia.

Simultaneous EMG was recorded by using surface bipolar electrodes (Medicotest pregelled child electrocardiogram-electrodes), placed over the right trapezius muscle halfway between the spinous process of the C7 vertebra and the acromion. The centre-to-centre interelectrode distance was 2.0 cm. The reference electrode was placed over the spinous process of C7. The EMG, as well as the LDF, signals were simultaneously displayed on an oscilloscope for monitoring electrode and probe function. All determinations were performed in a quiet laboratory room and at a temperature of 20–22° C.

Signal processing. The experimental setup is shown in the block diagram in Fig. 1. For determination of LDF, a laser-Doppler flowmeter was used (modified Periflux Pfld, Perimed, Stockholm, Sweden; time constant 0.2 s and 4 kHz, gain 1). The flowmeter building block constituted a photodetector, a high-pass filter, an

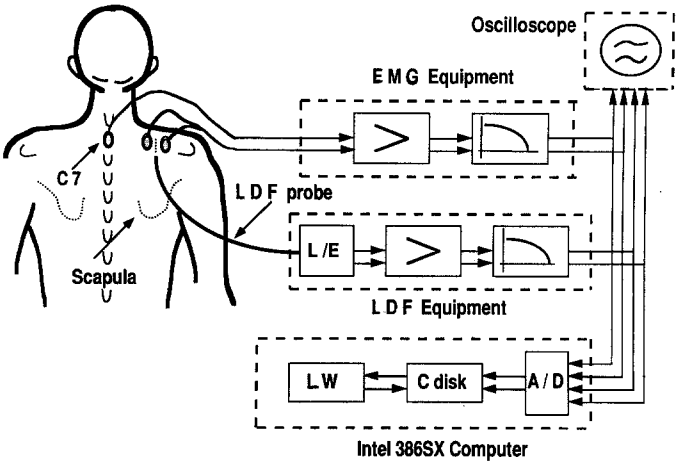


Fig. 1. Block-diagram of recording and analysis system for laser Doppler flowmetry (*LDF*) and electromyography (*EMG*). Amplifier; filter; *L/E* laser electronic signal transfer; *LW*, Lab-Window software

amplifier and a filter network with the transfer function. The LDF and EMG signals were converted into digital form in an A–D converter (AT-MIO-16, National Instruments, USA) with a resolution of 12 bits and processed on line by computer (Intel 386 SX/20 MHz processor, 140 MB hard disk). Fast Fourier transform from the time domain to the frequency domain was performed using Lab-Windows software. The EMG signals were preamplified and amplified using an EMG amplifier. The signals were filtered to avoid disturbing effects using an upper cut-off frequency of 1000 Hz and, for comparison, lower cut-off frequencies of 2 and 10 Hz. The root mean square (rms) signal, mean power frequency (MPF) and, for comparison, median spectrum frequency (MDF) were calculated using segments of 0.5 s. For each 1-min examination period we used 20 segments representing the 40th–50th s with exclusion of the first and the last segments to avoid disturbances from sample processing. In total, 18432 points were used per measurement. The LDF was calculated for each consecutive 1-min examination period using the last 20 s of each period. Before filtering, 2048 points·s^{-1} were used and after filtering 4 points giving a total of 80 points·measurement^{-1}. A frequency range of 0–8.2 Hz was used. The MPF and MDF were calculated according to Basmajian and DeLuca (1985) by using the following equations:

$$\text{for MPF,} \quad MPF = \frac{\int_0^\infty f W(f)\,df}{\int_0^\infty W(f)\,df}$$

$$\text{for MDF,} \quad \int_0^{MDF} W(f)\,df = \int_{MDF}^\infty W(f)\,df$$

where f is frequency; $W(f)$ is power spectrum density of the EMG signals.

Normalization of EMG and LDF and calculation of MPF and MDF. The LDF and rms-EMG were calculated in the time domain. The observed variability between individuals was relatively large both for EMG and LDF. Therefore, normalization was performed to reduce the influence of disturbances during the measurements and to facilitate comparisons between individuals. For LDF and rms-EMG all data were divided by the mean of the different measurement values of each individual test series, i.e. the values recorded at 0° (rest) and at 30, 60, 90 and 135° of arm elevation. Normalization was not used for MPF and MDF.

Statistical analyses. Multiple regression analyses were performed according to Neter et al. (1989). In the graphic presentation of the results, linear regression analyses of group mean values (Snedecor

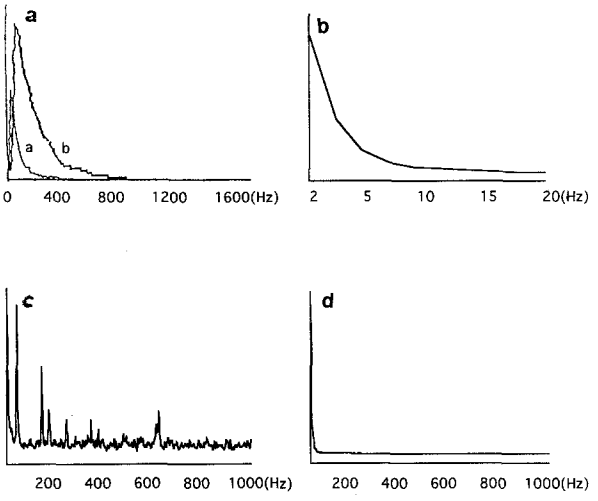
and Cochran 1967) were used. A value of $P<0.05$ was considered significant.

Results

LDF and EMG signals

The power spectrum density of LDF and EMG is shown in Fig. 2a–d and the power spectrum density of the unprocessed signals of LDF is shown in Fig. 2a. In comparison with the blood flow in the rest position with arms hanging (curve a), a rise in signal power (curve b) can be seen due to an increase in the blood flow of the trapezius muscle occurring during a sustained high force contraction brought about by keeping the arms elevated at 90° and carrying a 2-kg load in each hand. Doppler signals may also be generated by internal tissue motion other than blood flow. Muscle movement may be transferred to the optical fibre which gives a so-called motion artefact well-known from the laser-Doppler literature (Shephard and Öberg 1990). Analysis of the signal frequency power spectrum that was obtained at low and high force static contractions (Fig. 2a–b, above) showed that there were no noteworthy disturbances caused by different muscle fibre activity during low and high muscle strain. The frequency range 0–8.2 Hz was found to correspond to the blood flow most selectively (Fig. 2b). This range was used for LDF, a digital Butterworth low-pass filter of the 8th order being used in our programme.

The power spectrum of the processed EMG-signals is shown in Fig. 2c and d. The recordings performed at the rest position showed a mixture of different frequencies



Figs. 2a–d. The power spectrum density of laser-Doppler flowmetry (LDF) (**a** and **b**) and electromyography (EMG) (**c** and **d**). As regards LDF, the power spectrum density of the unprocessed signals is shown in **Fig. 2a.** In comparison to the signals recorded at rest (curve *a*), an increase in signal power (curve *b*) can be seen due to the increased microcirculation induced by a sustained high-force contraction of the trapezius muscle examined. **Figure 2b** shows the processed power spectrum of LDF. **Figure 2c** and **d** show the power spectrum of root mean square-EMG at muscle rest and at low-force contraction by arm elevation of 30°

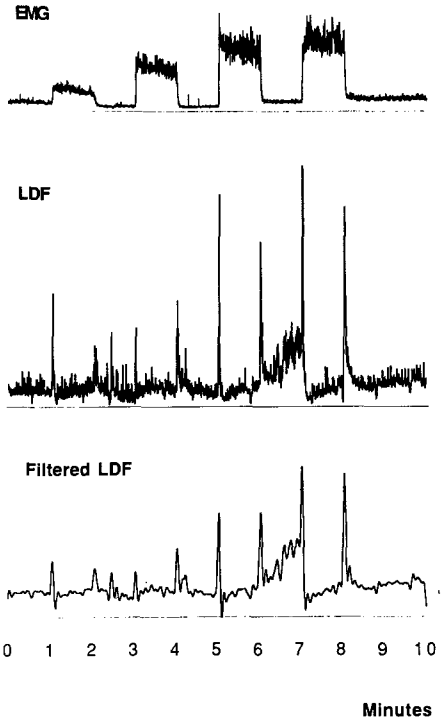


Fig. 3. Typical recordings of the root mean square (rms) converted *LDF* and *EMG* signals from one of our subjects. The LDF recordings are shown before and after processing of the signals. Rhythmic variations in the microcirculation with 5–6 cycles·min^{-1} (vasomotion) can be seen. Internal motion of the muscle during raising as well as lowering of the arms caused motion artefacts of the *LDF* during the first 5–10 s of each examination period. The *LDF* was measured during the last 20 s of each period and rms-EMG during the 40th to 50th s segment of the period. For definitions see Fig. 2

in the range 2–1000 Hz (Fig. 2c), while at 30° of arm elevation the main energy was within the low frequency range (Fig. 2d).

Typical LDF and EMG recordings from one of our subjects are shown in Fig. 3, as well as the effect of the processing in the Butterworth low-pass filter. At the beginning of each 1-min period, there is a motion artefact caused by the internal motion of the muscle occurring during the elevation as well as the lowering of the arms. Therefore, LDF was calculated for the last 20 s of each period.

Vasomotion

Rhythmic variations in muscle blood flow, vasomotion, were regularly recorded at 5–6 cycles·min^{-1}, as well as the small arterial (cardiac) pulsations of the microvascular flow (Fig. 3). These were consistently recorded at constant muscle activity with quite a few disturbances besides the background noise. The recording of these active rhythmic variations in small vessel flow would indicate that there were no noteworthy disturbances to the microcirculation from the presence of the optical fibre inside the muscle or from the trauma resulting from the insertion of the fibre.

Effect of the tourniquet

A tourniquet was applied to the left upper arm while LDF was recorded in the deltoid and brachioradial muscles (Fig. 4). When the blood circulation of the arm was arrested the LDF signals from the brachioradial muscle disappeared, as seen in the upper two recordings shown before and after processing of the signals, while LDF of the deltoid muscle above the tourniquet remained unchanged (lower recording).

Relationship of rms-EMG and LDF to arm-shoulder angle

The rms-EMG of the upper trapezius muscle showed a significant increase with increasing shoulder angle in contrast to LDF that showed no significant changes (Fig. 5). The highest values for both EMG and LDF were obtained at arm elevation of 135°. This was certainly due to the high tension and shortening of the muscle that is necessary for the rotation of the scapula in this position.

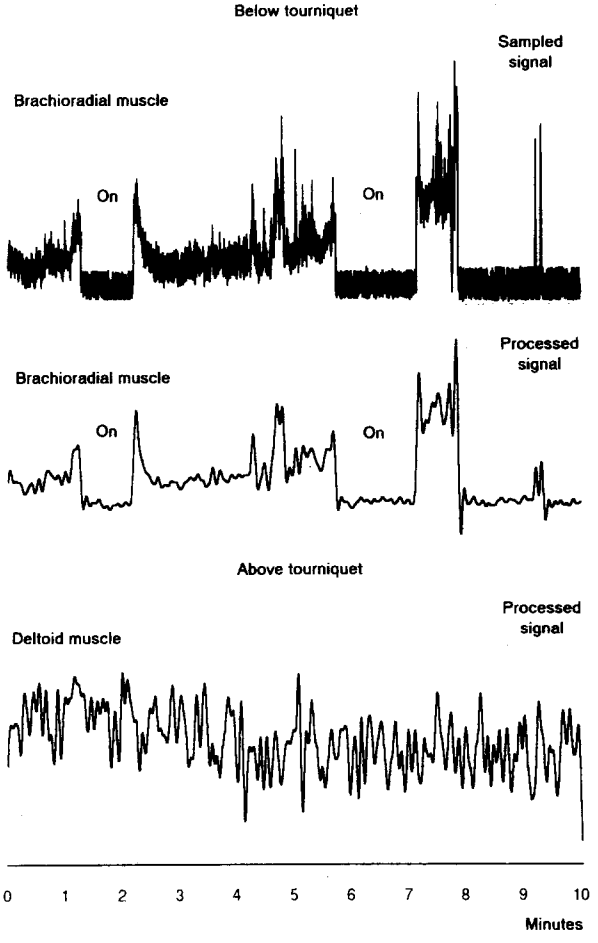


Fig. 4. Effect of a tourniquet applied to the upper arm while laser-Doppler flowmetry (LDF) was recorded in the deltoid and brachioradial muscles. When the blood circulation to the arm was stopped the LDF signals from the brachioradial muscle disappeared, as seen in the *upper two recordings* shown before and after processing of the signals, while LDF of the deltoid muscle above the tourniquet remained unchanged (*lower recording*)

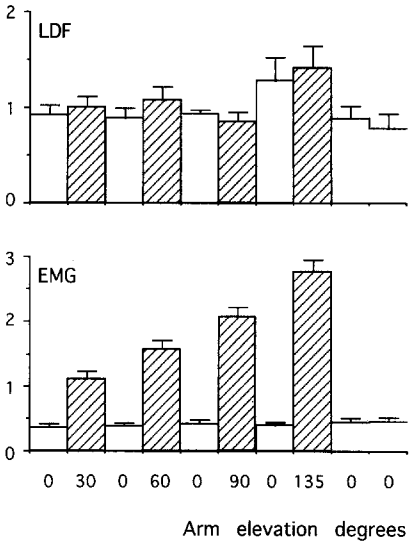


Fig. 5. The *LDF* and rms-*EMG* of the upper trapezius muscle in ten healthy men. The *EMG*, in contrast to *LDF*, shows a significant increase with increasing arm-shoulder angle (varying degree of arm elevation). The *LDF* showed no significant changes in this series. Means and SEM are given. For definitions see Fig. 2

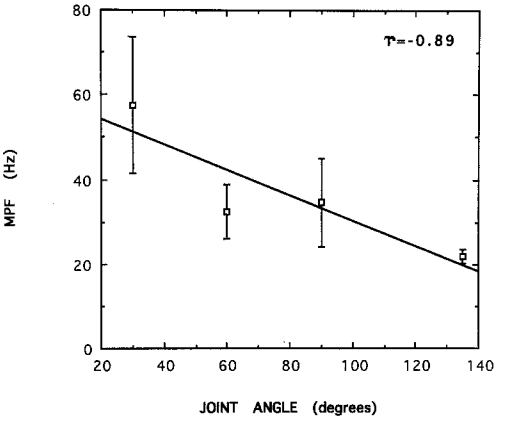


Fig. 6. The mean power frequency (*MPF*) showing a significant decrease with increasing arm-shoulder angle as a sign of local muscle fatigue

Relationship of MPF to arm-shoulder angle

In the frequency domain, we compared MPF and MDF both at the frequency rage 2–1000 Hz and the range 10–1000 Hz. The MPF showed large variability, although lower at 2–1000 Hz than at 10–1000 Hz, and MDF somewhat less variability at all contraction levels. The MDF showed the lowest variability for the frequency range 10–1000 Hz with coefficients of variation of the order of 8%–24%. The MPF at 2–1000 Hz is used from here onwards.

The MPF showed a significant decrease with increasing arm-shoulder angle indicating accumulated local fatigue (Fig. 6).

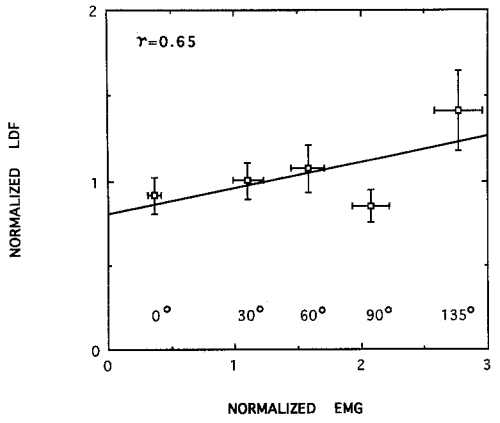


Fig. 7. Normalized *LDF* in relation to normalized *EMG* in the upper portion of the trapezius muscle. For definitions see Fig. 2

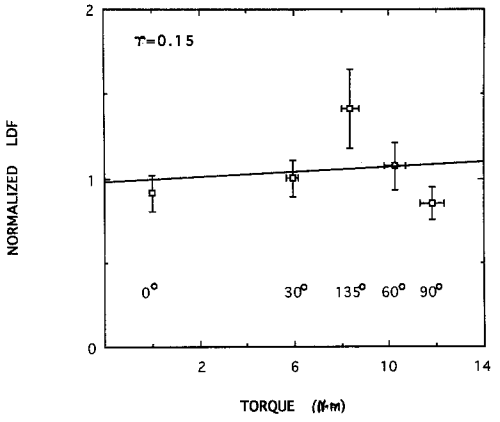


Fig. 8. Normalized laser-Doppler flowmetry (*LDF*) and shoulder torque showed a curvilinear relationship due to the high values of *LDF* at 135° of arm elevation (moderate torque)

The relationship of LDF to rms-EMG, shoulder torque and MPF

Linear regression of group data for normalized LDF in relation to normalized EMG recorded in the upper portion of the trapezius muscle at varying shoulder angle is shown in Fig. 7. The LDF showed an almost significant ($r = 0.65$) increase with increased muscle tension (EMG) brought about by increasing the elevation of the arms. A tendency to low values was, however, noted at a shoulder angle of 90°.

Multiple regression of all individual data indicated significant relationships between LDF and EMG and between LDF and shoulder torque. This was curvilinear with the highest values of LDF recorded at the moderate torque prevailing at 135° of arm elevation (Fig. 8), a position that necessitated considerable shortening (tension) of the upper trapezius muscle to keep the scapula rotated. At the higher torques at the shoulder angles of 60 and 90°, lower values of LDF were recorded with the lowest values being obtained at 90° of arm elevation. No significant relationship was obtained between LDF and MPF in this series.

Discussion

This study demonstrated that percutaneous single-fibre LDF can be performed clinically and gives further support to the findings presented by Tyml et al. (1990). The microcirculation in skeletal muscle was recorded dynamically during varying degrees of sustained static contraction and interposed rest.

Simultaneous EMG for the determination of the prevailing muscle activity was found to be necessary for more precise interpretation of the LDF recordings. This put high demands on signal processing and, therefore, on-line treatment of data using a 386SX computer was performed. The results were processed within 30 min after the recordings had been finished.

A light, continuous pressure on the optic fibre was used to maintain direct contact between probe and muscle during the whole examination procedure. Extreme movements like raising or lowering of the arms for changing the position to the one to be examined caused an initial motion artefact in the optic fibre during each 1-min period of load or rest. This did not influence the results since, for the recordings of LDF, only the last 20 s of each period were used and for EMG the 40th–50th s segment. It seemed that 5–10 s were needed to get stabilized conditions for the blood flow recordings. Our analyses of the power-spectrum density of LDF at low (rest) and high contraction force demonstrated an increase in the flux of red cells, i.e. numbers and velocities, at high force contraction with no disturbances from Doppler signals generated by internal tissue motion. The Periflux instrument used had an upper cutoff frequency of 4000 Hz so that movements with velocities higher than those of the blood cells were not recorded.

The LDF like the EMG showed relatively large between-individual variations. For LDF, spatial variations could easily have occurred due to the varying proximity of the probe to the larger arteries. So far, we have not examined the degree of variability for this factor. Our measurements included a hemisphere of the tissue with a radius of about 1 mm. This can be considered as a representative sample of the microvascular tree but spatial variations are likely to have occurred depending upon the actual position of the probe. Furthermore, spatial heterogeneity of blood flow and flow reserve has been found to be common in striated muscle (see Hargreaves et al. 1990; Menger et al. 1992).

A comparison between the magnitude of changes in the LDF and EMG signals at increasing load is of great interest. The EMG amplitude has generally been considered to indicate a state of tension of the contractile element which could be used as a relative measure of muscle metabolism (see Basmaijan and DeLuca 1985). With an unimpaired blood flow, energy can be renewed and waste products can be removed. The loads resulting from the 30, 60 and 90° of straight arm elevation caused a stepwise increase of the mean EMG activity of 200%, 300%, 400% and 600% that of the preceding rest period. In comparison, mean LDF showed much less of a change; at 30° of elevation there was an increase of 10%, at 60° an increase of 20%, at 90° a decrease of

10% and at 135° an increase of 10%. Our observation of a decrease in MPF could be considered to indicate accumulated local muscle fatigue. This was certainly reflected also by the 40% increase in the muscle blood flow that was observed during the rest period following the 90° of arm elevation. It would appear that the recruitment of motor units for maintained static contraction is much more efficient than the recruitment of capillaries necessary for the achievement of a sufficient, compensatory increase in microcirculation to meet the metabolic needs.

Local factors, such as the intramuscular pressure, might impede microcirculation during sustained static contraction. Our results would indicate that the upper portion of the trapezius muscle can maintain an elevated blood flow during sustained contraction to stabilize the scapula during maintained elevation of the arms. However, at 90° of arm elevation a tendency towards low LDF values was observed, possibly due to high intramuscular pressure induced by the relatively high shoulder torque. For the trapezius muscle relatively low intramuscular pressures have been reported for the majority of the positions examined, the 90° elevation possibly being an exception (Järvholm et al. 1991).

In conclusion, simultaneous LDF and EMG was used clinically for dynamic measurements of the microcirculation in relation to muscle tension at varying levels of static contraction and interposed rest. It would appear that biological variables, in the main cause large variations and therefore that at least ten individuals and test variations are needed so that regression analyses of the results can be performed.

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