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## Calf and shin muscle oxygenation patterns and femoral artery blood flow during dynamic plantar flexion exercise in humans

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**Abstract** The effects of dynamic plantar flexion exercise [40, 60, and 80 contractions·min<sup>-1</sup> (cpm)] on calf and shin muscle oxygenation patterns and common femoral artery blood flow ( $\dot{Q}_{fa}$ ) were examined in six female subjects [mean age 21 (SD 1) years] who exercised for 1 min at 33% of their maximal voluntary contraction at ankle angles between 90° and 100°. Spatially resolved near-infrared spectroscopy was used to measure medial gastrocnemius, lateral soleus (synergist) and anterior tibialis (antagonist) muscle oxygen saturation ( $SO_2$ , %).  $\dot{Q}_{fa}$  was measured by ultrasound Doppler. The  $SO_2$  changed significantly only in the medial gastrocnemius and its decrease (up to about 30%) was independent of the contraction frequencies examined. The increase in  $\dot{Q}_{fa}$ , at the end of exercise, was highest at 80 cpm. When the exercise at 60 cpm was prolonged until exhaustion [mean 2.7 (SD 1.1) min], medial gastrocnemius  $SO_2$  decreased, reaching its minimal value [mean 30 (SD 10)%] within the 1st min, and had partially recovered before the end of the exercise with concomitant increases in total haemoglobin content and  $\dot{Q}_{fa}$ . These results suggest that the medial gastrocnemius is the muscle mostly involved in dynamic plantar flexion exercise and its oxygen demand with increases in contraction frequency and duration is associated with an up-stream increase in  $\dot{Q}_{fa}$ .

**Keywords** Muscle oxygen saturation · Muscle metabolism · Near-infrared spectroscopy · Non-invasive measurements · Leg blood flow

### Introduction

The possibility of studying local muscle oxidative metabolism non-invasively during exercise has been recently enhanced thanks to the combination of <sup>31</sup>P-nuclear magnetic resonance spectroscopy (<sup>31</sup>P-NMRS) and near-infrared spectroscopy (NIRS) (Binzoni et al. 1998; Boushel et al. 1998; Mancini et al. 1994; Tran et al. 1999). NIRS has been largely used for investigating muscle oxidative metabolism in pathophysiology. NIRS uses light in the wavelength range 700–1,000 nm to transilluminate large sections of tissue. The high attenuation of light in tissues is due to:

- Oxygen-dependent absorption from chromophores of variable concentrations, i.e. haemoglobin (Hb), myoglobin (Mb), and cytochrome oxidase
- Absorption from chromophores of fixed concentration (skin melanine)
- Light scatter

In a scattering medium like tissue NIRS signal quantification is difficult and different methods have been proposed, the most reliable being spatially resolved spectroscopy (SRS; for review see Delpy and Cope 1997). In SRS, the slope of light attenuation compared to distance is measured at a distant point from the light input, from which the absolute ratio of oxy-Hb ( $O_2Hb$ ) to the total Hb content and hence average tissue saturation ( $SO_2$ ) can be calculated using the photon diffusion theory. The  $SO_2$  reflects the balance between  $O_2$  delivery and  $O_2$  utilisation in the tissue volume investigated. NIRS is a non-invasive and relatively low cost technique that offers the advantage of being less restrictive than <sup>31</sup>P-NMRS with regard to muscle performance and more comfortable and suit-

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able for monitoring, with high temporal resolution (up to 10 Hz), of multiple muscle groups. Its advantages and limitations (due to the difficulties with signal quantification, interference of high adipose tissue thickness and a controversial contribution of Mb to the NIRS signal) have been extensively reviewed (Boushel and Piantadosi 2000; Ferrari et al. 1997; McCully and Hamaoka 2000).

During exercise, both blood flow and oxidative metabolism in skeletal muscle respond to meet increased oxygen demand (Saltin et al. 1998). The literature is scarce on the relationship between up-stream blood flow and local muscle  $SO_2$  measured by NIRS in the exercising limb (Boushel et al. 1998; Hicks et al. 1999).

The triceps surae muscle, a principal ankle joint plantar flexor, is composed of three muscles, i.e. lateral gastrocnemius, medial gastrocnemius, and soleus. The contribution of the triceps surae plantar flexion torque is about 80% (Kawakami et al. 2000). The three muscles seem to function as synergists, but they differ in fibre type composition and structural properties. The gastrocnemii have similar proportions of types I and II fibres, whilst soleus, and tibialis anterior (the antagonist) have about 90% of type I muscle fibres (Jakobsson et al. 1990; Tamaki et al. 1998). It has been reported that, during repetitive maximal isometric contractions of the ankle plantar flexor muscles with the knee fully extended, the decrease in activation was higher in the gastrocnemius than in the soleus muscle, suggesting a greater susceptibility of the gastrocnemius to fatigue during plantar flexion (Kawakami et al. 2000). On the other hand, the lateral gastrocnemius seems to be inactive at ankle angles between 90° and 120° at 10% maximal voluntary contraction (MVC; Tamaki et al. 1997).

Differences in the energy metabolism of the gastrocnemius and soleus muscles during isometric voluntary continuous contractions were found by  $^{31}P$ -NMRS (Ratkvcicius et al. 1998). Tran et al. (1999) investigated the time course of the human gastrocnemius muscle phosphocreatine by  $^{31}P$ -NMRS, Mb/Hb desaturation by  $^1H$ -NMRS and continuous wave NIRS during a 4 min plantar flexion exercise at 60 cpm. The gastrocnemius maximal desaturation, as measured by NIRS, was reached 1.5 min after the onset of the exercise and tended to increase during the remaining part of the exercise.

The aim of this study was to investigate during dynamic plantar flexion exercise at one-third MVC:

1. The effect of contraction frequency [40, 60, and 80 contractions·min<sup>-1</sup> (cpm)] upon the oxygenation patterns (measured as  $SO_2$ ) of the medial gastrocnemius and the soleus, considered the two main ankle extensor synergists, and the tibialis anterior as their antagonist
2. The effect of all-out exercise at 60 cpm on the  $SO_2$  pattern of the medial gastrocnemius, considered to be the most involved and fatigable muscle

3. The relationship between  $SO_2$  patterns and femoral artery blood flow ( $\dot{Q}_{fa}$ ).

We hypothesised that during 1 min plantar flexion exercise these three muscle groups would show different  $SO_2$  patterns and each pattern in turn would be affected by contraction frequency. It is reasonable to suppose that the medial gastrocnemius muscle would be much more engaged in dynamic plantar flexion exercise than the soleus and the tibialis anterior showing the most consistent desaturation pattern; the latter would be enhanced, if the increase in  $O_2$  delivery to the muscle did not meet the increase in  $O_2$  utilisation during the exhausting exercise.

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## Methods

### Subjects

Six female subjects [mean age 21 (SD 1) years], who participated in this study, were physically active, although none were engaged in daily or intensive training programmes. Prior to the experiment, informed consent was obtained from each subject.

### Protocol

The subjects were familiarised with the test procedures to avoid simultaneous undesired movements of other body parts. The study was performed in a climatically (temperature and humidity) controlled chamber. The details of the experiment setup have previously been described (Kagaya 1992). All experiments were performed with the subjects in a supine position on a foot ergometer with the knee joint fully extended. The subjects placed the right foot (dominant leg) on the pedal of the ergometer with the ankle joint at 90°. The right thigh was at heart level and the lower leg at an upward angle of 10–15° to avoid venous pooling of blood. The leg was supported above the knee so that there was no contact between the muscle groups examined and the table, and the circulation to the leg was completely unrestricted. The MVC of the plantar muscle flexion was determined isometrically by averaging three of five trials, excluding the highest and the lowest values. The MVC test was performed 1 day before the beginning of the study itself to avoid any interference with the execution of the protocol. The subjects pressed the pedal with the ball of the foot to extend the ankle joint to 100°, then relaxed to return it to 90°, at frequencies of 40, 60 and 80 cpm, and with a constant load (one-third of that corresponding to the MVC of each subject: 71.7, 97.9, 72.4, 57.7, 106.8, 113.7 kg). Timing was made audible using a metronome. The subjects were asked to make a plantar flexion as correctly as possible and to keep constant the duty cycle characterised by 50% contraction and 50% relaxation. The execution of the exercise was accurately controlled for its duration. The subjects performed the same protocol consisting of three periods of 1 min plantar flexion exercise at 40 cpm, 60 cpm, and 80 cpm. The interval between periods was at least 15 min. At the end of the protocol the subjects were requested to exercise to exhaustion at constant load (one-third of MVC) and at 60 cpm (the frequency associated with the highest blood flow; Kagaya 1992). The same protocol was performed three times on separate days, and at the same time of day, to investigate the  $SO_2$  kinetics in all three muscle groups. Pen-marks were made on the skin to indicate the margins of the optical probes, to check for any sliding of the probe during the exercise, and to facilitate the repositioning of the probes on the following days.

## Cardiovascular responses

The cardiovascular responses were observed by monitoring heart rate (HR), mean arterial blood pressure (MABP), and common  $\dot{Q}_{fa}$  of the exercising leg. The HR and arterial oxygen saturation ( $S_aO_2$ ) were measured using pulse oximetry (Oxypal, Nihon Kohden, Tokyo, Japan). The finger cuff of a photoplethysmographic non-invasive arterial pressure monitor (Finapres, Ohmeda 2300, Englewood, Colo., USA) was placed on the middle finger of the left hand to measure beat-to-beat arterial pressure. The hand was supported in a sling at the level of the right atrium to eliminate hydrostatic pressure effects. Finapres measurements were initially corroborated using standard measurements of arterial pressure with an oscillometric cuff on the upper arm. The  $\dot{Q}_{fa}$  (in millilitres per minute) was calculated from integrated velocity during each cardiac cycle, vessel diameter and HR as previously described in detail (Kagaya and Homma 1997). In this study  $\dot{Q}_{fa}$  was measured instead of the popliteal blood flow because the subjects were performing the exercise in the supine position. Blood velocity was measured using Doppler ultrasound (HP Sonos 1000, USA) with a 5.6 MHz transducer. The gate length was kept at 8.9 mm. Two-dimensional recording was obtained by using a 7.5 MHz transducer with an ultrasound imaging system (HP 77030 A, USA) to estimate the diameter of the common femoral artery. Measurements were made on the common femoral artery before its branch. The height of the signal reflected the velocity of the blood flow (Kagaya and Homma 1997). A holder fixed the transducer at the selected position, so that manual correction of its direction was performed only when slight movement had occurred. Doppler signals were recorded and stored on videotape. The integrated velocities, during one cardiac cycle obtained by tracing the Doppler spectrum, were measured for three to five cycles during the pre-exercise control period and every six during exercise. Immediately after stopping exercise, the integrated velocities were measured for five cardiac cycles. Electromyography (EMG) was used as a marker for medial gastrocnemius contraction/relaxation (50%) and blood flow velocity analysis (Kagaya and Homma 1997). Bipolar surface electrodes (8 mm in diameter) were attached to the surface of the skin overlaying the right distal medial gastrocnemius. The EMG signals were amplified, transferred to the HP Sonos 1000 and, together with the Doppler signals, recorded simultaneously on a tape recorder for  $\dot{Q}_{fa}$  analysis. The contraction and relaxation times were determined by measuring the duration of the discharges and the time between electrical discharges, respectively. The contraction and relaxation ratio of the medial gastrocnemius was almost constant throughout the protocols. The EMG could not be measured on the other selected muscle groups (soleus and tibialis anterior) because the size of the optical probes did not allow the simultaneous use of surface EMG electrodes.

## Muscle oxygenation

Muscle oxygenation was measured using a three-wavelength (727, 803 and 827 nm) SRS based photometer (prototype of the OM-200, Shimadzu, Tokyo, Japan). The theory behind the near-infrared SRS method and the features of this device have previously been reported (Homma and Kagaya 1997; Matcher et al. 1995; Tsunazawa et al. 1996). The  $O_2Hb$  and deoxyhaemoglobin (HHb) changes in arbitrary units (a.u.) were collected every 2.85 s. Then, muscle  $SO_2$  and total haemoglobin content changes (tHb, a.u.) were calculated as  $O_2Hb/tHb \cdot 100$  and  $O_2Hb + HHb$ , respectively. The NIRS techniques were unable to differentiate between the amount of oxygen released by Hb and Mb because the absorbency signals of these two chromophores overlap in the near-infrared range. Few data are available on Mb concentration in the human leg; Mb is on average  $4 \text{ mg} \cdot \text{g}^{-1}$  wet tissue or  $41.9 \text{ } \mu\text{mol} \cdot \text{l}^{-1}$  in gastrocnemius (Mancini et al. 1994) and  $4.5 \text{ mg} \cdot \text{g}^{-1}$  in vastus lateralis (Masuda et al. 1999). Considering a calf blood volume of about 10% one can estimate its mass as a confounding factor at 20% of the whole Hb signal and it was assumed that most of the NIRS signal reflected changes in absorption of  $O_2Hb$  and HHb. There-

fore the  $SO_2$  value reflected predominantly the mean of arteriolar, capillary, and venular Hb  $SO_2$ . However, considering that different  $P_{50}$  of Hb and Mb can interfere differently with the NIRS signal according to the exercise intensity (for a review see Conley et al. 2000) the contribution of Mb could potentially limit the accurate interpretation of the  $SO_2$  signal. The tHb is related to changes in the total volume of Hb in the muscle region of interest.

The reliability of SRS in measuring  $SO_2$  has recently been evaluated both in vitro on tissue-like phantoms, and in vivo during forearm ischaemia (Suzuki et al. 1999). An excellent correlation was found between  $SO_2$  and data from co-oximetry in vitro, and between  $SO_2$  and muscle oxygen saturation data obtained in vivo by time resolved spectroscopy.

In order to position the optical probes, B-mode ultrasonography was used (Echo Camera SSD-500, Aloka Co, Tokyo, Japan) to localise each muscle group (medial gastrocnemius, lateral soleus, and anterior tibialis). The same apparatus was also used to measure the thickness of the adipose and muscle tissues (Homma et al. 1996). The adipose tissue thicknesses were 7 (SD 1), 6 (SD 1) and 6 (SD 1) mm in the medial gastrocnemius, lateral soleus and anterior tibialis muscles, respectively. The optical probes were firmly attached to the skin overlaying the thickest portion of the medial gastrocnemius, lateral soleus and anterior tibialis muscle groups [18 (SD 3), 14 (SD 2) and 25 (SD 2) mm, respectively]. In the case of the soleus muscle, the distal part was chosen to avoid the contribution of the lateral gastrocnemius muscle to the  $SO_2$  measurements.

## Statistics

Analysis of variance with repeated measures was used to test changes in  $\dot{Q}_{fa}$  and  $SO_2$ . Differences between pre-exercise and during/post-exercise measurements were detected by using Bonferroni  $\alpha$ -corrected paired *t*-test (BMDP2 V Statistical Software, Inc., Los Angeles, Calif., USA). To reduce the number of comparisons in multiple comparison tests, data were arranged according to the following scheme: the 20 pre-exercise data were averaged; the first 6 data during exercise were untreated; the following data during exercise were grouped every 5 s; the first 6 data after the end of exercise were untreated; the following ones during the recovery phase were grouped every 5 s.

## Results

The physiological parameters measured before and after each period of exercise are shown in Table 1. The HR had increased significantly at the end of a 1 min exercise performed at each contraction frequency, and at the end of the exhausting exercise. The increase was independent of contraction frequency. The MABP increased significantly only at the end of 1 min of exercise at 60 cpm, and at the end of the exhausting exercise. The  $S_aO_2$  was unchanged at the end of each period of exercise.

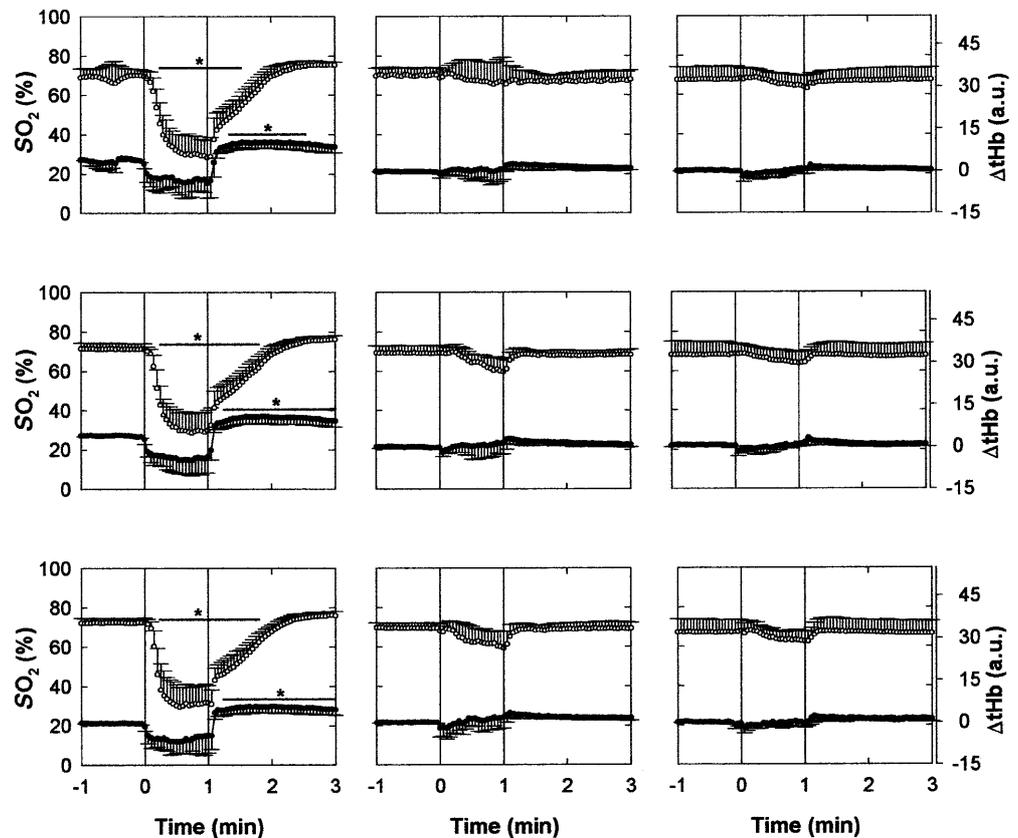
Muscle  $SO_2$  patterns of medial gastrocnemius, lateral soleus and anterior tibialis muscles during 1 min exercise at each contraction frequency are shown in Fig. 1. Pre-exercise  $SO_2$  of medial gastrocnemius, lateral soleus and anterior tibialis muscles were 69 (SD 5), 69 (SD 4) and 68 (SD 6)%, respectively (means of 1 min recording before exercise at 40 cpm). Considering that the three muscle groups have a similar thickness of adipose tissue, their  $SO_2$  values were compared but the results were not statistically significant. The  $SO_2$  and tHb of lateral soleus and anterior tibialis muscles were not affected

**Table 1** Values for heart rate (*HR*), mean arterial blood pressure (*MABP*) and arterial oxygen saturation ( $S_aO_2$ ) ( $n=6$ )

	40 Contraction·min <sup>-1</sup>		60 Contraction·min <sup>-1</sup>		80 Contraction·min <sup>-1</sup>		60 Contraction·min <sup>-1</sup> until exhaustion									
	Baseline		End exercise		Baseline		End exercise		Baseline		End exercise					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD				
HR (beats·min <sup>-1</sup> )	53	5	69*	9	55	6	73*	10	57	6	76*	10	59	7	80*	11
MABP (kPa)	10.0	1.9	11.9	1.9	10.3	2.3	12.3*	2.1	11.2	1.9	12.5	2.3	11.5	1.6	15.3*	2.1
$S_aO_2$ (%)	98	1	99	1	98	2	98	1	98	1	98	1	99	1	98	1

\* $\alpha=0.05$  Compared to baseline

**Fig. 1** Time course of muscle oxygen saturation ( $SO_2$ ; ○) and changes in total haemoglobin (*tHb*; ●) in the medial gastrocnemius (*left panels*), lateral soleus (*central panels*) and anterior tibialis (*right panels*) muscles during one-third maximal voluntary contraction (*MVC*) exercise at 40 (*upper row*), 60 (*central row*) and 80 (*lower row*) contraction·min<sup>-1</sup>. The *vertical lines* represent the duration of the exercise ( $n=6$ ). Significance was set at  $\alpha=0.05$ . \*Significant compared to pre-exercise

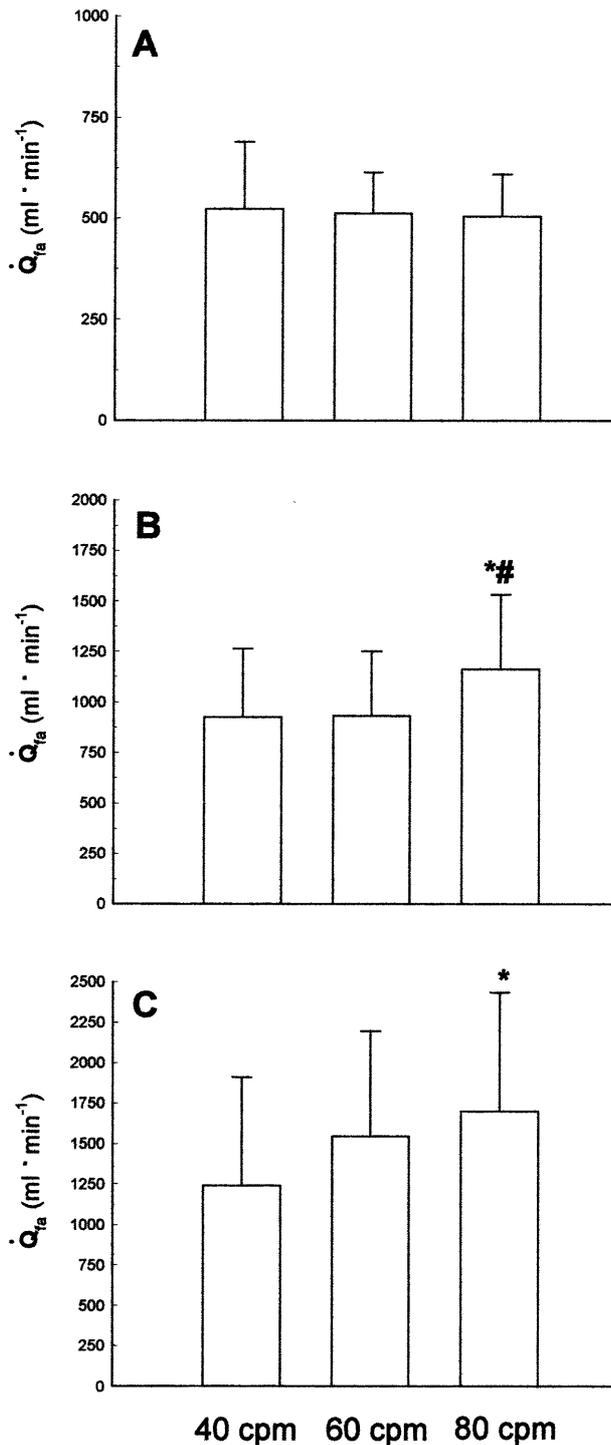


significantly by exercise performed at any of the contraction frequencies tested. Conversely,  $SO_2$  of medial gastrocnemius started to decrease significantly about 15 s after the onset of each period of exercise reaching a minimal value (approximately 30%) about 15 s later. In considering the difference between the end of each exercise period and the respective pre-exercise value, the desaturation of the medial gastrocnemius was similar at the three contraction frequencies. The *tHb* decreased, although not significantly, in the medial gastrocnemius during all three periods of exercise. The  $SO_2$  of the medial gastrocnemius had recovered to the baseline value within 32 s and 45 s after the end of the 40 cpm, and 60 and 80 cpm exercises, respectively. The *tHb* of the medial gastrocnemius was significantly higher than the baseline at about 20 s after the end of the 40 cpm

exercise, and was back to its baseline value about 70 s later. The *tHb* increased significantly from 14 s after the end of both 60 and 80 cpm exercise, without returning to the corresponding baseline within the 2 min observation period.

The  $\dot{Q}_{fa}$ , over 1 min exercise, increased immediately after the onset of exercise at each contraction frequency with a large inter-individual variability. At the end of exercise, a significant difference in  $\dot{Q}_{fa}$  was found between 40 and 80 cpm, and between 60 and 80 cpm (Fig. 2). Immediately after the end of exercise, a significant difference in  $\dot{Q}_{fa}$  was found only between 40 and 80 cpm.

The time course of medial gastrocnemius muscle  $SO_2$  and changes in *tHb* during exhausting exercise at 60 cpm are shown in Fig. 3. The mean time to



**Fig. 2** Relationship between common femoral artery blood flow ( $\dot{Q}_{fa}$ ) and contraction frequency (contraction·min<sup>-1</sup>, cpm) before exercise (A), at the end of exercise (B), and immediately after the end of exercise (C) ( $n=6$ ). Significance was set at  $\alpha=0.05$ . \*Significant compared to 40 cpm, # significant compared to 60 cpm

exhaustion was 2.7 (SD 1.1) min. In six subjects  $SO_2$  dropped within 15 s of the start of exercise, reaching the minimal value [30 (SD 10)%] within the 1st min of exercise. After the 1st min of exercise,  $SO_2$  was almost

constant up to exhaustion in two subjects (D, F), whilst in the remaining four subjects  $SO_2$  gradually increased. The tHb decreased promptly at the beginning of the exercise more clearly in four subjects (A, B, C, E) and gradually recovered, being higher than baseline values at the end of the exhausting exercise. After the end of the exhausting exercise,  $SO_2$  increased slowly to reach the baseline values. In some subjects,  $SO_2$  was still higher than its baseline value 2 min after the end of exercise. The  $\dot{Q}_{fa}$  and  $SO_2$  measured before exercise, at 1 min exercise, at the time of exhaustion, and immediately after the end of exercise ( $\dot{Q}_{fa}$  only) are shown in Fig. 4. With reference to the pre-exercise value,  $\dot{Q}_{fa}$  increased after 1 min exercise and increased further either at the end or immediately after the cessation of the exercise;  $SO_2$  was significantly lower at 1 min exercise and at the end of exercise. Moreover,  $SO_2$  at the time of exhaustion was significantly higher than that found at 1 min exercise.

## Discussion

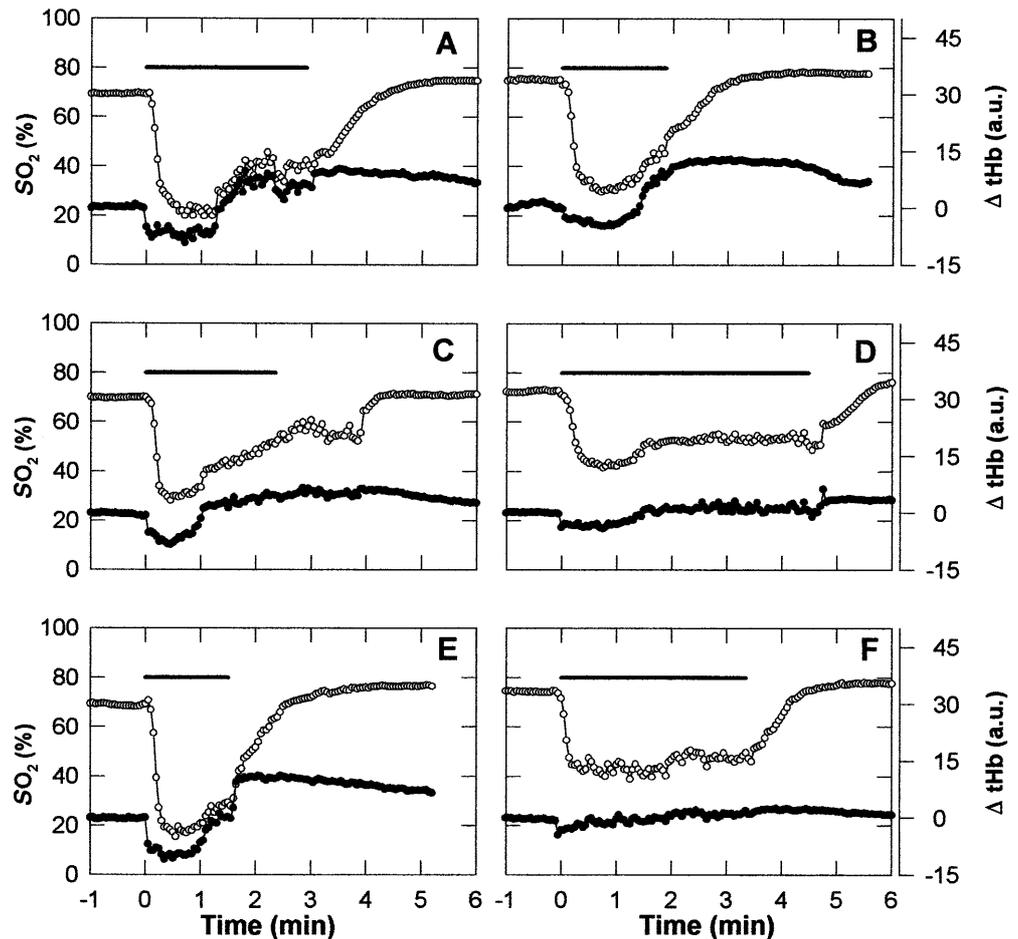
The principal findings of this study were:

1.  $SO_2$  decreased during 1 min dynamic plantar flexion exercise (at ankle angles between 90 and 100°) with the knee fully extended both in the two main ankle synergists (medial gastrocnemius and lateral soleus) or in the antagonist (anterior tibialis) muscles at all contraction frequencies tested. However, the magnitude of desaturation was significant only in the medial gastrocnemius, the most engaged muscle group of the triceps surae muscles according to EMG findings (Tamaki et al. 1997). Moreover, there was no contraction frequency effect on  $SO_2$  of the medial gastrocnemius, thus not supporting the stated hypothesis. The increase in  $\dot{Q}_{fa}$  at the end of 1 min exercise was highest at 80 cpm.
2. In all-out exercise medial gastrocnemius  $SO_2$  decreased, reaching its minimal value within the 1st min of exercise, and unexpectedly tended to increase during the remaining part of the exhausting exercise in four out of the six subjects. Concomitantly  $\dot{Q}_{fa}$  increased progressively from 1 min to the cessation of exercise.

## Blood flow and dynamic plantar flexion exercise

The regulation of the blood flow to human skeletal muscle during exercise has recently been reviewed (Delp and Laughlin 1998; Saltin et al. 1998). In the transition from rest to exercise there is a rapid initial elevation in blood flow. Depending on the intensity of the exercise, the blood flow level is stabilised within 30 to 90 s. Calf blood flow, measured (by strain gauge plethysmography) after dynamic plantar flexion exercise, increased as contraction frequency increased and reached a peak at 60–80 cpm (Kagaya 1992). In this study  $\dot{Q}_{fa}$  changes (measured by

**Fig. 3** Time course of  $SO_2$  (○) and changes in  $tHb$  (●) in the medial gastrocnemius muscle for each subject (A–F) during one-third maximal voluntary contraction exercise to exhaustion at 60 cpm. The horizontal line represents the duration of the exercise. For definitions see Fig. 1



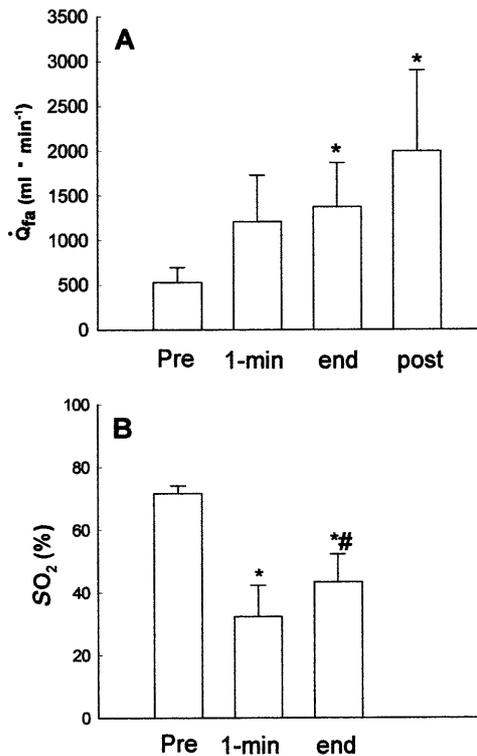
ultrasound Doppler) during plantar flexion exercise reflected changes in blood flow occurring only in the lower leg because the upper leg was inactive. The reproducibility of  $\dot{Q}_{fa}$  measurements during dynamic exercise using ultrasound Doppler has been reported by several investigators and recently reviewed (Saltin et al. 1998). In our study, as expected,  $\dot{Q}_{fa}$  increased during 1 min of exercise performed at 33% MVC and the highest value was found in association with the greatest amount of work done (80 cpm) (Fig. 2). During exhausting exercise at 60 cpm  $\dot{Q}_{fa}$  increased after 1 min and significantly at the end of exercise (Fig. 4). The lack of significance in the increase of  $\dot{Q}_{fa}$  at 1-min 60 cpm (Figs. 2, 4) could also be attributable to the variability among subjects and their low number. The further rise in  $\dot{Q}_{fa}$  immediately after the cessation of the exercise (Fig. 4), not accompanied by a further consistent increase in  $tHb$  (except subject E; Fig. 3), might be attributable to the absence of a mechanical constraint.

#### Muscle $SO_2$ measurements

The principles of NIRS have previously been reported (Delpy and Cope 1997). Near-infrared light penetrates skin, subcutaneous fat and underlying muscle, and is either absorbed or scattered within the tissue. A detector responds to part of the scattered light. The influence of

the thickness of the adipose tissue layer on light propagation in leg muscles has recently been investigated; light penetrates shallow regions of muscle under the skin and subcutaneous fat even at an adipose tissue thickness of 1.5 cm (Matsushita et al. 1998). Since the thickness of the adipose tissue in the subjects examined was about 7 mm, the  $SO_2$  signal reflected the metabolic changes occurring mainly in the muscle tissue.

Although, Tran et al. (1999) recently demonstrated by  $^1H$ -NMRs that oxy-Mb desaturation kinetics matches the NIRS signal, the potential Mb contribution to the NIRS signal has been described in the Methods. The kinetics and the amount of the Mb desaturation during constant load calf exercise are controversial (for review see Conley et al. 2000). Conflicting results were also found between muscle oxygenation by NIRS and venous blood oxygen saturation in normoxia but not in hypoxia (Hicks et al. 1999; MacDonald et al. 1999). In those studies, muscle oxygenation was measured using two wavelength continuous wave systems. The relative scale was obtained using the differential NIRS signal between rest and after an ischaemic occlusion of the limb for 10 min. This method is inaccurate and its limitations have been recently discussed in details (McCully and Hamaoka 2000). The recent introduction of the near-infrared SRS instrumentation in muscle exercise physiology (Boushel et al. 2000) allows a more quantitative



**Fig. 4** **A** Common femoral artery blood flow ( $\dot{Q}_{fa}$ ) during one-third maximal voluntary contraction exercise to exhaustion at 60 contraction·min<sup>-1</sup> (cpm) measured before exercise (*pre*), at the end of 1 min of exercise, at the end of exercise, and immediately after the end of exercise (*post*). **B** Muscle oxygen saturation ( $SO_2$ ) during 60 cpm exhausting exercise measured before exercise, at the end of 1 min of exercise, and at the end of exercise ( $n=6$ ). Significance was set at  $\alpha=0.05$ . \*Significant compared to pre-exercise, # significant compared to the end of 1 min of exercise

and accurate approach, measuring  $SO_2$  as a percentage. The  $SO_2$  represents the saturation in the microvasculature of muscle tissue. The medial gastrocnemius muscle  $SO_2$  values (at rest) in this study were comparable with those obtained by Boushel et al. (2000). The differences in medial gastrocnemius and vastus lateralis muscles oxygenation patterns during a standardised treadmill test, and the different recovery times from exercise-induced desaturation in the quadriceps muscles have already been studied (Chance et al. 1992; Quaresima et al. 1998). To the best of our knowledge, we are the first to report the desaturation patterns of the calf and shin muscle groups during 1 min dynamic plantar flexion exercise at different contraction frequencies (Fig. 1). Although  $SO_2$  decreased in all the muscle groups investigated, the magnitude of the decrease was significant only in the medial gastrocnemius and it was unaffected by the different contraction frequencies. The tHb of the medial gastrocnemius decreased during all three periods of exercise. These changes were not statistically significant for the variability of the signal and/or the low number of the subjects; however, they do not influence the interpretation of  $SO_2$  changes because  $SO_2$  takes into account the changes in tHb. The consistent desat-

uration found in the medial gastrocnemius reflects the imbalance between the oxygen demand and supply to this muscle group that is predominantly involved in this kind of exercise. The expected further reduction of the  $SO_2$  value at the greatest work done (80 cpm) was probably compensated by the increase in  $\dot{Q}_{fa}$ . In the other two muscle groups examined, the oxygen supply was adequate to meet the oxygen demand. The different  $SO_2$  patterns amongst the three muscle groups may also reflect their differences in fibre type composition and levels of intracellular metabolites (Achten et al. 1990; Jakobsson et al. 1990; Ratkevicius et al. 1998; Rico-Sanz et al. 1999; Tamaki et al. 1998). Although  $SO_2$  recovered in about 30 s after the end of each exercise bout, tHb increased significantly about 15 s after the end of the exercise without returning to its baseline value after exercise at 60 and 80 cpm (Fig. 1). The prolonged tHb rise reflects the local post-exercise compensatory vasodilatation and release of intramuscular pressure. The hyperaemic response, due mainly to locally released factors, does not match the up-stream changes in  $\dot{Q}_{fa}$  (Bangsbo and Hellsten 1998).

The level and the pattern of desaturation of the medial gastrocnemius muscle as well as  $\dot{Q}_{fa}$  were affected significantly by all-out exercise (Figs. 3, 4). The partial resaturation was also accompanied by a progressive increase in tHb (subjects A, B, C, E). In these subjects tHb was higher than the baseline values before the end of exhausting exercise suggesting an increase of local blood volume in the muscle. Skin vasodilatation might result in an increase in tHb, even if the contribution of skin overlaying muscle depends on the optode separation distance which has been shown to be around 5% of the total oxygenation changes provoked by arterial occlusion (Boushel and Piantadosi 2000). The partial resaturation before the end of exercise could be explained by a variety of factors mostly of a local nature e.g., the increase in oxygen delivery to the muscle rather than an increased local oxygen extraction from the circulating haemoglobin to meet the further demand for oxygen (Grassi et al. 1999), or myoglobin (NIRS detectable) resaturation. Other explanations could be the recruitment of different fibre types within the muscle volume investigated, skin vasodilatation for heat dissipation, blood flow redistribution amongst muscle groups, or different involvement of other muscle groups. The increase in tHb and  $\dot{Q}_{fa}$  could suggest that an increase in local blood flow to the medial gastrocnemius muscle can be considered the main cause of the anticipated beginning of the recovery of  $SO_2$ .

In conclusion, these results indicate that during 1 min of dynamic plantar flexion exercise at one-third MVC the oxygenation, measured as  $SO_2$ , decreases more markedly in the medial gastrocnemius than in the synergistic muscle (soleus) and in the antagonist (anterior tibialis) muscle at any contraction frequency (40, 60 and 80 cpm) and confirm the previous findings based on the main involvement of the medial gastrocnemius during plantar flexion at ankle angles between 90° and 100°. In

addition, the anticipated resaturation of the medial gastrocnemius before the end of the all-out exercise can be mainly attributed to the increase in the local blood flow as measured indirectly by the increase in the tHb and the up-stream  $\dot{Q}_{fa}$ .

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