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ORIGINAL ARTICLE

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Muscle metabolism from near infrared spectroscopy during rhythmic handgrip in humans

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Abstract The rate of metabolism in forearm flexor muscles ($\dot{M}O_2$) was derived from near-infrared spectroscopy (NIRS- O_2) during ischaemia at rest rhythmic handgrip at 15% and 30% of maximal voluntary contraction (MVC), post-exercise muscle ischaemia (PEMI), and recovery in seven subjects. The $\dot{M}O_2$ was compared with forearm oxygen uptake ($\dot{V}O_2$) [$\text{flow} \times (\text{oxygen saturation in arterial blood} - \text{oxygen saturation in venous blood}, S_aO_2 - S_vO_2)$], and with the ^{31}P -magnetic resonance spectroscopy-determined ratio of inorganic phosphate to phosphocreatine ($P_i:PCr$). During ischaemia at rest, the fall in NIRS- O_2 was more pronounced [76 (SEM 3) to 3 (SEM 1)%] than in S_vO_2 [71 (SEM 3) to 59 (SEM 2)%]. During the handgrip, NIRS- O_2 was lower at 30% compared to 15% MVC [58 (SEM 3) vs 67 (SEM 3)%] while the S_vO_2 was similar [29 (SEM 3) vs 31 (SEM 4)%]. Accordingly, $\dot{M}O_2$ as well as $P_i:PCr$ increased twofold, while $\dot{V}O_2$ increased only 30%. During PEMI after 15% and 30% MVC, NIRS- O_2 fell to 9 (SEM 1)% and “0”, but the use of oxygen by forearm muscles was not reflected in S_vO_2 . During reperfusion after PEMI, the peak NIRS- O_2 was lowest after intense exercise, while for S_vO_2 the reverse was seen. The discrepancies between NIRS- O_2 and S_vO_2 , and therefore between the estimates of the metabolic rate, would suggest significant limitations in sampling venous blood which is representative of the flexor muscle cap-

illaries. In support of this contention, S_vO_2 and venous pH decreased during the first seconds of reperfusion after PEMI. To conclude, NIRS- O_2 of forearm flexor muscles closely reflected the exercise intensity and the metabolic rate determined by magnetic resonance spectroscopy but not that rate derived from flow and the arterio-venous oxygen difference.

Key words Exercise · Energy production · Magnetic resonance spectroscopy · Venous oxygen saturation

Introduction

The signal obtained by near infrared spectroscopy of skeletal muscle (NIRS- O_2) has been assumed to reflect the combined absorption of light by haemoglobin (Hb) and myoglobin (Mb) depending upon their state of oxygenation, thereby representing the balance between the oxygen supply and consumption (Chance et al. 1988; 1992). Oxygen saturation in venous blood (S_vO_2) was also been regarded as a reflection of this balance (Severinghaus 1994) and a relationship between these two variables is likely. During rhythmic handgrip Mancini et al. (1994) have reported such a correlation and Wilson et al. (1989) have found a similar relationship during electrical stimulation of muscles in the dog. Costes et al. (1996) have confirmed the correlation between NIRS- O_2 and S_vO_2 during cycling in conditions of hypoxia, but a significant correlation was not found in normoxia. During cycling, NIRS- O_2 of the quadriceps muscles has been shown to decrease with exercise intensity to reach a plateau at maximal intensity which was not exceeded by cuff-induced ischaemia, indicating complete desaturation (Chance et al. 1992). It has been suggested that changes in oxyhaemoglobin (HbO_2) may be more pronounced than in oxymyoglobin (MbO_2) (Richardson et al. 1995) except during complete anoxia or ischaemia (Wang et al. 1990). Interpretation of S_vO_2 measurements for comparison with NIRS- O_2 is contingent upon obtaining blood samples which are representative of the capillaries and this

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has been shown to be especially uncertain during ischaemia (Deblasi et al. 1992).

The purpose of this study was to compare values of muscle oxygen saturation and consumption derived from measurements of NIRS, S_vO_2 with blood flow, and ^{31}P magnetic resonance spectroscopy (^{31}P -MRS). Values were compared during ischaemia at rest, rhythmic handgrip, post-exercise muscle ischaemia (PEMI), and recovery. To assess if venous blood was representative of the capillary blood, care was taken to obtain samples immediately after flow was re-established during reperfusion of the forearm following release of an occlusion cuff on the upper arm. We hypothesized that the indices of the muscle metabolism would reflect the exercise intensity and determinations were made both at a moderate and a maximal intensity.

Methods

After informed consent, two healthy female and five healthy male subjects, aged 25–39 years, participated in the study as approved by the Ethics Committee of Copenhagen. For the measurements of ischaemia at rest, each subject was supine with the arm parallel to the body and the wrist in a neutral position. A cuff placed around the upper arm was inflated to 280 mmHg and maintained at that pressure for 10 min, while NIRS- O_2 and S_vO_2 were determined every minute. The cuff was then deflated and determinations were made immediately and every 5 s for the 1st min. Parallel experiments were conducted for ^{31}P -MRS on a separate day, and venous blood was also sampled to determine the reproducibility of the responses between the test days. The ^{31}P -MRS spectra were obtained every 30 s throughout the experiment.

Static handgrip strength was determined with a strain gauge dynamometer (Caspersen and Nielsen, Copenhagen, Denmark) as the highest of three maximal voluntary contractions (MVC). The 15% and 30% MVC intensities were derived from the MVC force of each subject. The handgrip task involved rhythmic contractions corresponding to the designated force (15%, 30% of static MVC force) which was recorded and displayed visually for each subject as a voltage. The hand was positioned parallel to the body in a neutral position while the subjects lay supine. The forearm was cushioned to alleviate extraneous muscle activity, and the handgrip device was suspended so that subjects held it passively. Rhythmic handgrip was performed to a force corresponding to 15% and 30% MVC at a cadence of 1 Hz for 2 min.

In the first experiment, NIRS- O_2 was recorded every 15 s, venous blood sampled every 30 s and arterial blood after 1 min. At 5 s before the end of exercise, the occlusion cuff was inflated and the PEMI interval initiated. During PEMI, NIRS- O_2 and venous blood were obtained every 15 s for 3 min. The cuff was then deflated and measurements were repeated as after ischaemia at rest.

After a further 20 min of recovery, the protocol was repeated at 30% MVC. Adequacy of the recovery periods was established by ensuring that the lactate concentration of venous blood and pH had returned to the level at rest [0.9 (SEM 0.01) and 7.38 (SEM 0.01) mmol · l⁻¹, respectively]. The protocol was repeated on a separate day for ^{31}P -MRS; and again for forearm blood flow by ultrasound Doppler and total Hb derived by NIRS. During the ^{31}P -MRS study, spectra were obtained every 30 s during handgrip, PEMI, and recovery. The NIRS-determined total Hb was determined every 15 s during handgrip and PEMI, and every 5 s during the recovery. Doppler velocity spectra and arterial diameter were determined continuously and flow calculated every 30 s during handgrip and PEMI, and every 5 s during the recovery. During the ^{31}P -MRS study, venous blood was also obtained to determine whether S_vO_2 differed between days.

Near infrared spectroscopy

An INVOS 3100 (Somanetic Corporation, Troy, MI) and a NIRO 500 (Hamamatsu, Japan) was used to determine muscle oxygen saturation. The optodes of the instrument were positioned over the prominence of the flexor digitorum superficialis muscle, located during passive extension of the wrist and voluntary grip contraction. The INVOS apparatus used light at 730 and 810 nm wavelengths to evaluate light attenuation in the tissue with a depth proportional to the separation between the optodes. The NIRS- O_2 saturation was calculated by enhancing the signal from deep tissues using the method described previously by Pollard et al. (1996). The optodes were placed at a distance of 3 and 4 cm, and with this separation it was expected that light would penetrate to the depth of the flexor digitorum profundus and carpi radialis muscles, all of which have been shown to engage in handgrip exercise (Mizuno et al. 1990; Van der Zee et al. 1992).

Changes in total Hb were calculated relative to the resting level from data obtained by the NIRO 500, which used four wavelengths with a photo-multiplier. The emitting and receiving optodes were placed 3.5 cm apart, and a differential pathlength factor of 3.59 for forearm muscle was used to account for the scattering of light in the tissue as proposed by Van der Zee et al. (1992).

Magnetic resonance spectroscopy

The ^{31}P -MRS was carried out with a Vivospec spectrometer (Otsuka Electronics) interfaced to a 80-cm long, 26-cm bore 2.9-T magnet (Magnex Scientific, Abingdon, UK). Each subject sat with the dominant arm placed in the magnet. A 4-cm diameter, two-turn inductively driven surface coil was placed in the position marked for NIRS and a non-magnetic strain gauge dynamometer (PFN Teknik, Copenhagen, Denmark) was held passively in the hand. Homogeneity of the magnetic field was optimized by shimming on the proton signal. Line width at the start was less than 0.5 parts per million. The ^{31}P -MRS spectra were obtained at 49.83 MHz by single 90° pulse excitations with pulse power and width optimized by the height of the phosphocreatine (PCr) peak. The PCr, inorganic phosphate (P_i), and γ adenosine triphosphate (ATP) spectra were collected in 2 000 points over 205 ms with 6 s interpulse delay. Data were acquired in 2-min blocks at rest and in 30-s blocks during exercise and recovery. The signal to noise ratio at rest ranged from 12 to 16. Before Fourier transformation, data were multiplied by 5-Hz exponential line broadening. Preceded by baseline correction, integration of peak areas was performed by a least squares method assuming Lorentzian line shape. This procedure has been shown to provide good agreement with biochemical evaluation of PCr and ATP concentrations in muscle biopsies (Bangsbo et al. 1993).

Blood sampling

A catheter was inserted towards the hand into a deep vein in the antecubital space. After 2 ml of blood had been discarded, a 1-ml sample was collected anaerobically in heparinized syringes and immediately placed on ice. The catheter was flushed with 1 to 2 ml of saline at rest and during handgrip, while during ischaemia, the exact volume of the catheter and extension line (0.8 ml) was used. For arterial blood, a catheter was placed in the brachial artery. The S_vO_2 and oxygen saturation in arterial blood (S_aO_2) were determined by haemoximetry (OSM3, Radiometer, Copenhagen, Denmark), pH by an ABL-3 apparatus (Radiometer), and lactate concentration by a YSI 2300 analyser (Yellow Springs, Ohio).

Ultrasound Doppler

An ultrasound Doppler (CFM 800, Vingmed, Horten, Norway), equipped with an annular phased-array transducer probe (Ving-

med) operating at an imaging frequency of 7.5 MHz, was used for imaging of the brachial artery (Rådegran 1997). The artery was insonated at a perpendicular angle. Its diameter was determined from two-dimensional (2-D) longitudinal image sections along the central path of the ultrasound beam, where optimal spatial resolution occurs. Systolic and diastolic diameters for each cardiac cycle were individually determined. A diameter corresponding to one third of the systolic and two thirds of the diastolic values ($\bar{D} = \bar{D}_{\text{systole}_{1/3}} + \bar{D}_{\text{diastole}_{2/3}}$) was used to represent of diameter over the cardiac cycle. A variable Doppler frequency of 4–6 MHz, operating in high-pulsed repetition frequency mode (4–36 KHz) was used for blood velocity measurements. The velocity was measured during real-time 2-D visualization of the artery. This procedure allowed centring and size adjustment of the sample volume across the diameter of the vessel and thus over the full spatial distribution of the velocity profile. A PC with an A-D converter allowed transfer and storage of the Doppler signals and heart rates determined from the electrocardiogram. The mean blood velocity (\bar{v}) was angle-corrected, time and space averaged, and amplitude weighted. Volume flow was calculated from the continuous \bar{v} and the arterial radius (r) as $\bar{Q} = \bar{v}\pi r^2$. At rest, during handgrip and PEMI, blood flow was determined from \bar{v} collected over ten cardiac cycles in the last 30 s of each minute, and over five cycles during recovery.

Calculations

Since the NIR signal has been shown only to provide relative changes due to the unknown optical pathlength in the tissue (Chance et al. 1988), the NIRS- O_2 data were normalized by defining the range between fully oxidized and reduced states for each subject as recommended by Chance et al. (1992) and Hamaoka et al. (1996). The highest value was observed during reperfusion after PEMI, and the lowest ("0") was obtained during PEMI after 30% MVC. The muscle metabolism (MO_2 ; $\mu\text{mol} \cdot \text{s}^{-1}$) was determined using the initial rate of NIRS- O_2 (%) desaturation during ischaemia (DeBlasi et al. 1992; Hamaoka et al. 1996), and applying a muscle oxygen store of 0.35 mmol (Hamaoka et al. 1996). Forearm oxygen consumption ($\dot{V}O_2$) was determined as the product of the blood flow in the brachial artery and the arterio-venous oxygen difference, of which SAO_2 [97 (SEM 1)%] never changed significantly.

Free adenosine diphosphate concentration [ADP] was calculated from the MRS data as $([Cr] \times [ATP \times 10^{-3}]) / ([PCr] \times [H^+] \times 1.66 \times 10^9)$ assuming a creatine kinase equilibrium, a rest ATP concentration of 5.5 mmol \cdot kg $^{-1}$ wet mass and a [PCr] \times ([PCr] + [Cr]) ratio at rest of 0.6. The pH was calculated according to Taylor et al. (1983) and in the case of P_i splitting (Mizuno et al. 1994), a weighted average of the P_i peaks was used.

During rhythmic handgrip, anaerobic ATP production was calculated as $\Delta PCr + 3/2(\beta \Delta pH + k \Delta PCr)$, where $k = 1/(1 + 10^{pH-6.75})$ and the buffer capacity was taken as 40 slykes as shown by Kemp and Radda (1994). It was assumed that the increase in H^+ was accounted for only by lactate production and that only an insignificant release of H^+ and lactate took place from muscle during the measurement.

The aerobic ATP production was calculated from forearm $\dot{V}O_2$ and NIRS-determined MO_2 assuming a phosphorus to oxygen ratio of 2.5. The ATP production was expressed as millimoles per kilogram of muscle assuming a forearm flexor volume of 300 ml (Winters and Woo, 1990) and a muscle density of 1.05 \cdot kg $^{-1}$.

Changes with time and between conditions were evaluated using the Friedman test and if found significant, such differences were located by Wilcoxon's test. Interdependencies were determined by Spearman's test. Also, to obtain the half-time responses ($t_{1/2}$), relationships were fitted by a least squares method. Differences were considered significant if P was less than 0.05.

Results

Ischaemia at rest

While S_vO_2 decreased during the initial 5 min [from 71 (SEM 3) to 59 (SEM 2)%], the decrease in NIRS- O_2 was much more pronounced, and it reached 3 (SEM 1)% (Fig. 1) and the two variables were correlated ($r = 0.6$; $P < 0.01$). The initial rate of reduction in NIRS- O_2 was by 0.27% \cdot s $^{-1}$ corresponding to a MO_2 of 1.3 $\mu\text{mol} \cdot \text{s}^{-1}$. The P_i :PCr rose after 4 to 5 min [from 0.13 (SEM 0.01) to reach 0.30 (SEM 0.01)] as did ADP [25 (SEM 1) to 34 (SEM 3) $\mu\text{mol} \cdot \text{kg}^{-1}$; Fig. 1]. Both muscle and venous pH remained at the values seen at rest [7.05 (SEM 0.02) and 7.38 (SEM 0.02), respectively].

Recovery

Upon release of the cuff, S_vO_2 decreased initially to 38 (SEM 2)% (Fig. 1). The subsequent recovery was with a

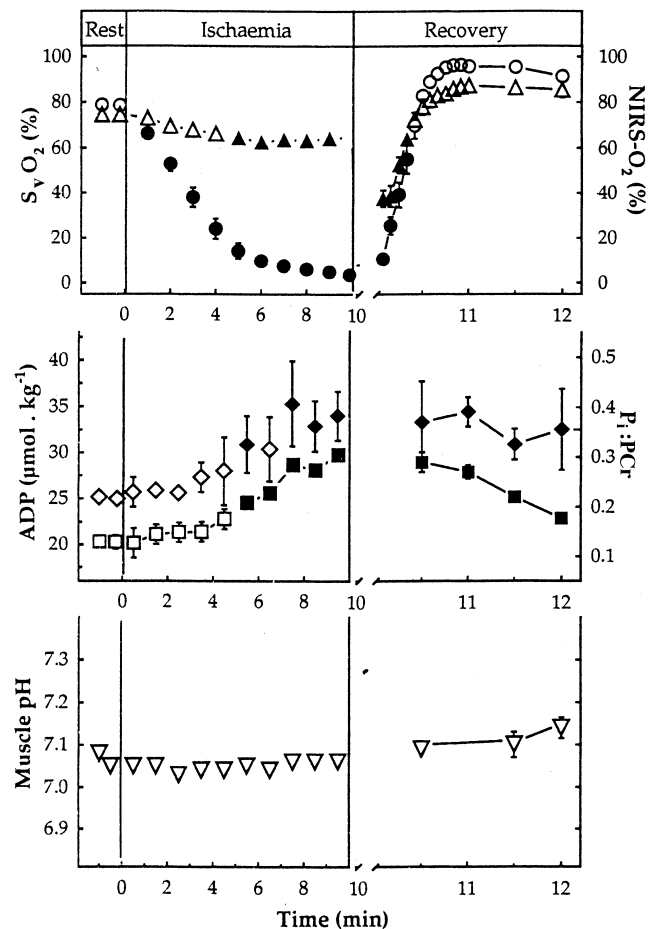


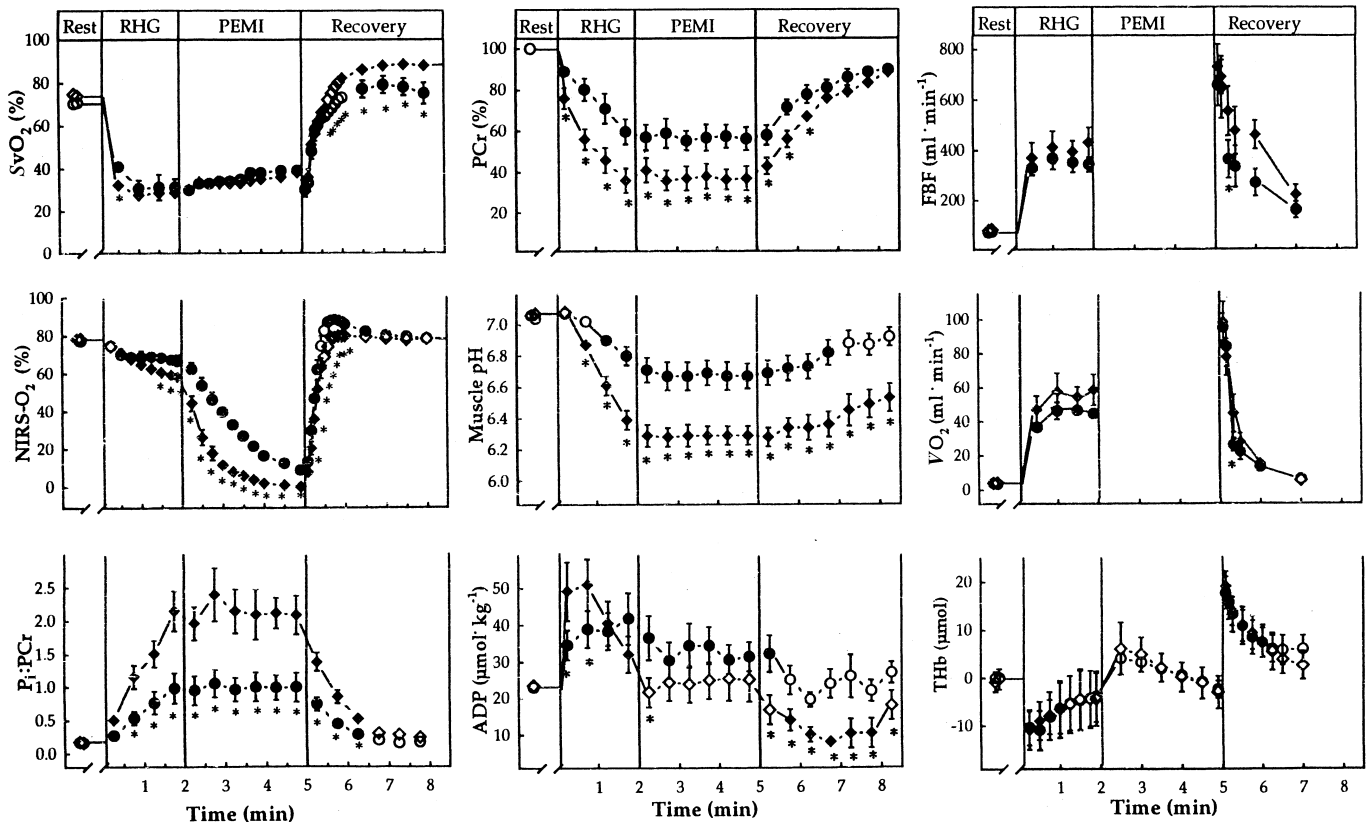
Fig. 1 Forearm metabolic variables at rest, during forearm ischaemia, and recovery. Values are mean and SEM NIRS- O_2 (circle). Near infrared spectroscopy of forearm flexor muscles, S_vO_2 (triangle) oxygen saturation of venous blood, P_i :PCr (squares) ratio of muscle inorganic phosphate to creatine phosphate, ADP (diamonds) adenosine diphosphate; muscle pH (inverted triangles). Filled symbol different from rest $P < 0.05$

$t_{1/2}$ of 13 (SEM 1s) and after 1 min, the peak value was 87 (SEM 1)% (16% above rest). Also, the NIRS- O_2 recovery had a $t_{1/2}$ of 13 (SEM 1) s and it had a peak of 97 (SEM 1)% (20% above rest) that coincided with the peak for S_vO_2 , and the two were correlated ($r = 0.82$; $P < 0.01$). The Pi:PCr recovered, while ADP did not reach the rest level. Venous and muscle pH did not change.

Rhythmic handgrip exercise at 15% maximal voluntary contraction

The S_vO_2 fell after 30 s, reached 31 (SEM 4)% after 1 min and then remained stable (Fig. 2). After 30 s, NIRS- O_2 also decreased [to 70 (SEM 3)%], and the decrease continued to an end value of 67 (SEM 3)%. The $t_{1/2}$ was 24 (SEM 6) and 15 (SEM 1) s, respectively, and the two variables were correlated ($r = 0.64$; $P < 0.01$).

Fig. 2 Forearm metabolic and blood flow variables at rest, during rhythmic handgrip exercise (RHG), post-exercise muscle ischaemia (PEMI), and recovery. Values are mean and SEM. Circles 15% maximal voluntary contraction (MVC) diamonds 30% MVC. S_vO_2 oxygen saturation, in venous blood, NIRS- O_2 near infrared spectroscopy determined O_2 saturation, Pi:PCr muscle inorganic phosphate to phosphocreatine ratio, PCr muscle phosphocreatine, ADP adenosine diphosphate, FBF forearm blood flow, $\dot{V}O_2$ forearm oxygen uptake, THb NIRS determined total haemoglobin. Filled symbol different from rest $P < 0.05$, * difference between intensities $P < 0.05$



The Pi:PCr reached 1.0 (SEM 0.2) and ADP rose to a plateau of 41 (SEM 5) $\mu mol \cdot kg^{-1}$ as PCr fell to 60 (SEM 6)% of the rest level (Fig. 3). Within 1 min, muscle pH fell to 6.8 (SEM 0.03); likewise, venous pH (after 90 s) fell to 7.30 (SEM 0.01). During the MRS study, the end-handgrip S_vO_2 was similar [30 (SEM 5)% vs 31 (SEM 4)] to the value on the previous day when NIRS- O_2 and S_vO_2 were determined.

Forearm blood flow increased from 77 (SEM 6) to 352 (SEM 39) $ml \cdot min^{-1}$ and $\dot{V}O_2$ from 4.0 (SEM 0.2) to 46 (SEM 3) $ml \cdot min^{-1}$ (Fig. 2). The mean brachial artery diameter ($1/3\text{Ø}$ systolic + $2/3\text{Ø}$ diastolic) was 4.4 (SEM 0.1) mm. Relative to rest, total Hb fell by 10 (SEM 4) μmol at the beginning of handgrip exercise and subsequently recovered 6 (SEM 5) μmol . The anaerobic ATP production was 99 (SEM 20) $mmol \cdot kg^{-1}$ and the aerobic production 52 (SEM 4) $mmol \cdot kg^{-1}$ (Fig. 3).

Post-exercise muscle ischaemia

Paradoxically, S_vO_2 increased throughout the ischaemic period to reach an end value of 39 (SEM 3)% (Fig. 2). In contrast, NIRS- O_2 fell to a nadir of 9 (SEM 1)% with a $t_{1/2}$ of 48 (SEM 2) s and the two variables did not correlate. The initial NIRS- O_2 rate of reduction was approximately twice that established during ischaemia at rest and MO_2 was $2.3 \mu mol \cdot s^{-1}$. The Pi:PCr, PCr, muscle pH, ADP, and venous pH all remained similar to the end-exercise values. The Hb first rose by 4 (SEM 1)

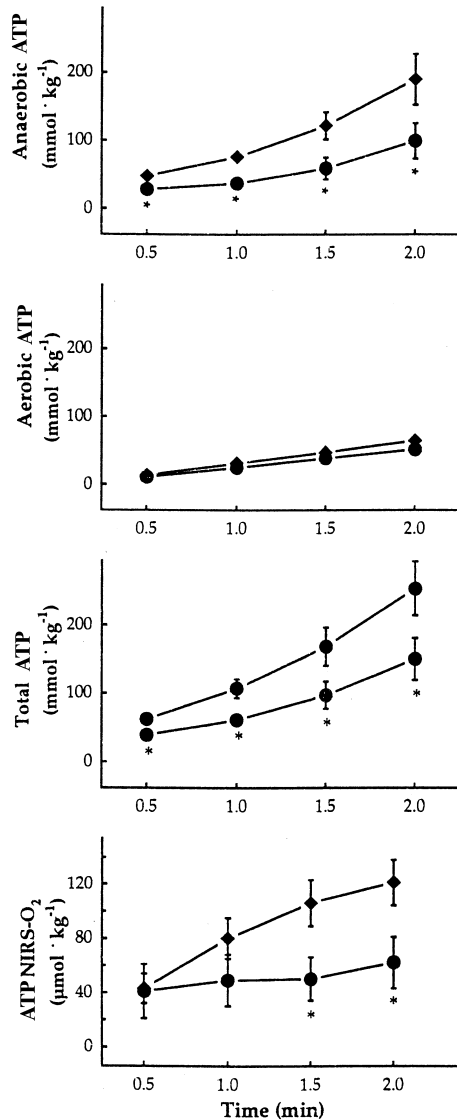


Fig. 3 Estimates of anaerobic and aerobic and anaerobic adenosine triphosphate (*ATP*) production; the sum of the two, and *ATP* turnover based on the near infrared spectroscopy (*NIRS*)-determined muscle metabolism for rhythmic handgrip exercise. Values are mean and SEM Circles 15% maximal voluntary contraction (*MVC*); diamonds 30% *MVC*. * Difference between intensities $P < 0.05$

μmol with cuff inflation, and then fell 2 (SEM 2) μmol as 18 ml of blood was withdrawn.

Recovery

The S_vO_2 decreased immediately after release of the cuff followed by a recovery with a $t_{1/2}$ of 15 (SEM 2) s (Fig. 2). The rest value was attained in 30 (SEM 3) s; and after 90 s it reached a peak of 78 (SEM 4)% corresponding to 7% above rest. For *NIRS-O₂* the recovery was with a $t_{1/2}$ of 15 (SEM 1) s to reach the value at rest in 30 (SEM 6) s and the peak of 88 (SEM 1)% appeared after 45 s (12% above rest). Thus, *NIRS-O₂* and S_vO_2 were correlated ($r = 0.79$; $P < 0.01$).

The *Pi:PCr* recovered with a $t_{1/2}$ of 27 (SEM 5) s (Fig. 2). The *ADP* returned to the rest value by 60 s and muscle *pH* recovered after 2.5 min. Immediately after release of the cuff, venous blood *pH*, as well as S_vO_2 , fell marginally, and then recovered towards the rest value (Figs. 2, 4).

In the first 5 s, blood flow rose to 659 (SEM 85) ml · min⁻¹ and then returned towards the rest level (Fig. 2). After 1 min, $\dot{V}O_2$ was 13.5 (SEM 2) ml · min⁻¹. The *Hb* rose to 17.6 (SEM 3) μmol above the rest level and it recovered after 1 min.

Rhythmic handgrip exercise at 30% maximal voluntary contraction

Within 30 s, S_vO_2 fell to 32 (SEM 3)%, reached 29 (SEM 3)% after 1 min, and then, as during 15% *MVC*, remained stable (Fig. 2). The $t_{1/2}$ was 9.0 (SEM 0.7) s and after 30 s it was not significantly different from the 15% *MVC* value. The *NIRS-O₂* fell to 70 (SEM 2)% after 30 s and reached 58 (SEM 3)% by the end of exercise; thus, it was lower than during 15% *MVC* in the last 90 s. The $t_{1/2}$ was 72 (SEM 7) s and *NIRS-O₂* was correlated to S_vO_2 ($r = 0.56$; $P < 0.01$).

The *Pi:PCr* was higher than at 15% *MVC* after 60 s and reached 2.2 (SEM 0.3) with a slope twice that at 15% *MVC* (Fig. 2). The *PCr* fell to 36 (SEM 6)% of the rest level. The rise in *ADP* was above the 15% *MVC* value [51 (SEM 5) μmol · kg⁻¹], but by the end of exercise, it approached the rest level. After 30 s, venous blood and muscle *pH* fell to 7.23 (SEM 0.02) and 6.38 (SEM 0.06), respectively (Figs. 2, 4). Again during the *MRS* study, the end-handgrip S_vO_2 was similar [28 (SEM 4)%] to the value [29 (SEM 3)%] on the previous day when *NIRS-O₂* and S_vO_2 were determined.

The flow was 431 (SEM 55) ml · min⁻¹ and the $\dot{V}O_2$ 58.6 (SEM 9) ml · min⁻¹ (Fig. 2). The *Hb* fell 10 (SEM 4) μmol below the level at rest and recovered 5 (SEM 5) μmol with no significant difference between the two exercise intensities. The anaerobic *ATP* production

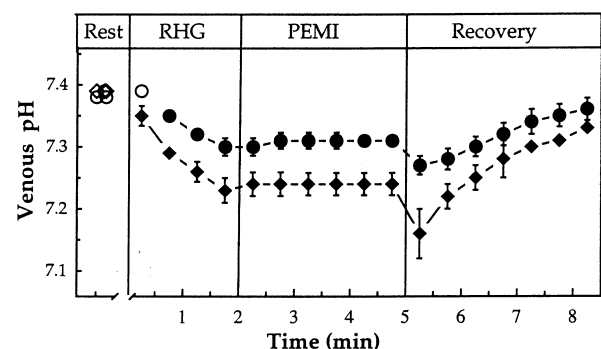


Fig. 4 Venous blood *pH* at rest, during rhythmic handgrip (*RHG*), post-exercise muscle ischaemia (*PEMI*), and recovery. Values are mean and SEM. Circles 15% maximal voluntary contraction (*MVC*), and diamonds 30% *MVC*. Filled symbol different from rest $P < 0.05$, * difference between intensities $P < 0.05$

reached 190 (SEM 37) $\text{mmol} \cdot \text{kg}^{-1}$, or twice the 15% MVC value (Fig. 3). The aerobic production was 64 (SEM 10) $\text{mmol} \cdot \text{kg}^{-1}$; only 25% higher than at the lower exercise intensity.

Post-exercise muscle ischaemia

As during 15% MVC, S_vO_2 increased to 38 (SEM 1)% (Fig. 2). In contrast, NIRS- O_2 decreased with a $t_{1/2}$ of 21 (SEM 2) s to reach "0", and the two set of data did not correlate. Thus, NIRS- O_2 was always lower than during 15% MVC and the initial decrease showed a slope twice ($\times 2.2$) that of 15% MVC with a MO_2 of $5.1 \mu\text{mol} \cdot \text{s}^{-1}$.

The Pi:PCr remained at the end-exercise value thus being higher than after 15% MVC (Fig. 2). Both PCr and ADP, as muscle and venous pH, were similar to the end-exercise values. The Hb increased 6 (SEM 5) μmol above the rest level with cuff inflation and subsequently fell to 3 (SEM 3) μmol below the rest level.

Recovery

The S_vO_2 recovered with a $t_{1/2}$ of 12 (SEM 1) s, the rest value was reached in 35 (SEM 2) s, and after this point it was higher than after 15% MVC. (Fig. 2). After 1 min, it was 14 (SEM 1)% above the level at rest [89 (SEM 1)%]. The NIRS- O_2 recovered with a $t_{1/2}$ of 15 (SEM 1) s and while it also reached the rest value in 30 (SEM 4) s, it did not exceed that level and was thereby lower than after 15% MVC. The correlation coefficient between S_vO_2 and NIRS- O_2 was 0.84 ($P < 0.01$).

The Pi:PCr recovered with a $t_{1/2}$ of 31 (SEM 4) s and it remained higher for 2 min than after both ischaemia at rest and 15% MVC PEMI (Fig. 2). Similarly, PCr recovered but it was lower than after 15% MVC for the first 90 s. The ADP decreased from min 1–3 of recovery and then returned to the rest level. Venous pH fell initially to 7.16 (SEM 0.04) and then, as with muscle pH, approached the level at rest. For all phases of the experiment, muscle and venous blood pH were correlated ($r = 0.8$; $P < 0.01$).

Blood flow rose to 730 (SEM 110) $\text{ml} \cdot \text{min}^{-1}$ and $\dot{V}O_2$ was 15 (SEM 2) $\text{ml} \cdot \text{min}^{-1}$ after 1 min (Fig. 2). The Hb increased to 19 (SEM 3) μmol above the rest level and then recovered after 1 min.

Discussion

This study compared two methods for studying patterns of oxygen saturation and metabolic rate during rhythmic handgrip exercise, PEMI, and recovery. We found changes in NIRS O_2 saturation of the forearm flexor muscles during handgrip consistent with MRS-determined metabolic rate, but not reflected in regional S_vO_2 and forearm $\dot{V}O_2$. Also, during both rest and PEMI, the kinetics of S_vO_2 and NIRS- O_2 were different: while S_vO_2

established a plateau, the NIRS signal continued to decrease.

During ischaemia at rest, NIRS- O_2 and S_vO_2 were correlated, but the fall in S_vO_2 was relatively small compared to NIRS- O_2 . During post-exercise muscle ischaemia, the two variables were not correlated and there was in fact a slight increase in S_vO_2 while NIRS- O_2 fell markedly. The common finding that S_vO_2 remained quite constant would indicate that the blood sampled came predominantly from the veins with little contribution from the capillaries, although the NIRS did detect a reduction of Hb with each blood withdrawal. A possible explanation for the slight increase in S_vO_2 during post-exercise muscle ischaemia is that the vascular bed of the forearm was provided with blood from the veins of the upper arm during the rapid inflation of the occlusion cuff, as suggested by the increase in total Hb. This blood would have been unlikely to have represented the metabolism in the forearm muscles, and the influence of translocated blood may have been more pronounced after exercise because the blood volume in the forearm would have been lowered by the muscle pump.

With unimpeded blood flow to the forearm during rhythmic handgrip exercise, and especially during reperfusion after PEMI, it is much more likely that S_vO_2 would have reflected saturation in the muscle capillaries and, in fact, the correlation between NIRS- O_2 and S_vO_2 became better the higher the flow: during rhythmic handgrip the r was 0.6 at a flow of $400 \text{ ml} \cdot \text{min}^{-1}$ and during reperfusion after PEMI, the r was 0.82 at a forearm flow of $700 \text{ ml} \cdot \text{min}^{-1}$.

During rhythmic handgrip exercise, it remains puzzling why S_vO_2 did not decrease from 15% to 30% MVC although flow was limited, but this pattern is in line with the results of Wahren (1976). This occurred even though the pH of blood was lower (7.23 vs 7.30) which would support desaturation upon the passage through the capillaries (Stringer et al. 1994). Thus, the unchanged S_vO_2 , despite a fall in pH, may indicate a diffusion limitation between the capillaries and forearm muscles. It could be that the forearm flexor muscles constituted so small a fraction of the forearm vascular bed that we were unable to detect the corresponding change. However, during handgrip exercise most of the forearm muscles have been shown to become engaged (Mizuno et al. 1994), especially at 30% MVC, making it unlikely that the venous blood sampled at the elbow was not influenced significantly by the muscle activity.

Furthermore, if the unchanged S_vO_2 reflected an admixture of blood from other less active tissues this would possibly have occurred also for venous pH. The fact that $\dot{V}O_2$, did the as flow, increased to a much higher level after the release of the upper arm cuff does not contradict an argument for a diffusion limitation. However, with reperfusion, $\dot{V}O_2$ represents only partly the metabolic rate as resaturation of both Hb and Mb would have been included in the value calculated. However, the resaturation of Mb requires diffusion of oxygen into the cell. Thus, either blood admixture or a lower Mb O_2 could

have explained the opposite patterns of peak NIRS- O_2 and S_vO_2 during recovery after 15% and 30% MVC.

The reduction in total Hb at the onset of exercise reflected the fact that the muscle pump directs blood towards the heart and the subsequent recovery towards the rest level during handgrip exercise that the muscle became hyperaemic. Furthermore, during the recovery from PEMI, the highest value for total Hb was noted as being concomitant with the highest flow being elicited in the passive forearm in response to *reperfusion hyperaemia*. During handgrip exercise, the forearm blood flow was only 55% of the value noted during reperfusion after PEMI, and almost identical at the two exercise intensities (352 and 431 ml · min⁻¹) which supports the suggestion that has been made of a circulatory limitation to $\dot{V}O_2$, even when the capacity of the heart is unlikely to be challenged (Kagaya and Ogita 1992). In humans involved in exercise with a larger muscle mass, a maximal flow rate has been reported only during arm cranking. In that situation, it has been reported that an increasing exercise intensity is accomplished by an increasing involvement of the muscles of the upper body (Secher and Saltin 1994). Compared to the exercise intensities normally used during, for example leg exercise, the 30% MVC, in fact represented a *maximal* effort, as the force achieved fell to 94 (SEM 2)% of the intended level in the last seconds of the handgrip.

One explanation for the lack of a substantial increase in blood flow from 15% to 30% MVC could be that flow was impeded by the mechanical forces of muscle contraction. It was apparent from the Doppler velocity spectra that flow to the muscle at 30% MVC occurred almost entirely during relaxation. Alternatively, forearm flow may have been limited by sympathetic vasoconstriction, which then was eliminated during the reperfusion. During rhythmic handgrip exercise Joyner et al. (1992) has argued for a higher forearm flow when the sympathetic outflow to the arm is blocked after application of local anesthetics to the stellate ganglion.

The NIRS apparatus was unable to distinguish between changes in saturation of Hb and Mb. Thus, a limitation of this study was that we were unable to determine the extent to which the discrepancies between NIRS- O_2 and S_vO_2 during handgrip were due to differences in Mb O_2 . Yet, the marked decrease in S_vO_2 during exercise (40%) with a relatively stable NIRS- O_2 (~20% reduction), as opposed to the stable S_vO_2 during PEMI with a drop in NIRS- O_2 to its lowest level (~60% lower than during handgrip), supports the suggestion that Mb O_2 becomes desaturated only during PEMI (Wang et al. 1990).

With no knowledge of the mechanical efficiency during rhythmic handgrip exercise, a central assumption of this study was that the forearm metabolic rate would increase in proportion to the exercise intensity. This has been established for MO_2 (Belardinelli et al. 1995) and for the MRS-determined ATP turnover, but this was not the case for forearm $\dot{V}O_2$. Thus, it was unique to NIRS- O_2 that it evaluated the aerobic contribution to metab-

olism. In confirmation of the results of Hamaoka et al. (1996), when the muscle metabolic rate was evaluated by MRS-derived indices of ATP turnover, we found a high correlation to MO_2 . Notably, the muscle metabolism derived from MRS suggested an approximate twofold increase from 15 to 30% MVC, as did the calculation based on NIRS- O_2 , but not the rate derived from blood flow and the arterio-venous difference in oxygen saturation. The finding that forearm blood flow was limited and S_vO_2 did not decrease in proportion to exercise intensity makes many of the premises for modelling muscle metabolism during exercise (Severinghaus 1994) invalid.

Forearm $\dot{V}O_2$ determined by the product of blood flow and the arterio-venous difference in oxygen saturation would suggest that *anaerobic* metabolism was prominent especially when the forearm was made ischaemic, both at rest and following rhythmic handgrip exercise. In that situation, NIRS- O_2 became reduced, while there was no change in muscle or in venous blood pH as indications of glycolysis. One conclusion could be that glycolysis is coupled to the mechanical events in the muscle during contraction, leading to the decrease in pH only during the handgrip exercise as found by Quistorff et al. (1993). Alternatively, the oxygen store in the capillary Hb O_2 , and in Mb O_2 in the muscle cells, could be large enough to provide for tissue metabolism during ischaemia after exercise was performed, precluding the need for glycolytic activity.

Towards the end of the exercise at 30% MVC, force was diminished (fatigue) with a comparable fall in anaerobic ATP production from 2.1 to 1.9-fold the 15% MVC value, signifying a maintained ATP production-to-demand ratio as found by Hogan et al. (1996). Free ADP fell markedly but apparently in a manner unrelated to ATP turnover. The decline in NIRS- O_2 and the maintained $\dot{V}O_2$ suggested that mitochondrial respiration may not have been strictly controlled by ADP as suggested by Barstow et al. (1994).

To conclude, NIRS- O_2 of forearm flexor muscles closely reflected the exercise intensity and the MRS-determined metabolic rate, but not the rate derived from blood flow and the arterio-venous difference in oxygen saturation. These findings illustrate the validity and potential advantages of NIRS- O_2 for measuring muscle oxidative metabolism during exercise and ischaemia.

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