Lactate and H^+ uptake in inactive muscles during intense exercise in man

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1. The present study examined how uptake of lactate and H^+ in resting muscle is affected by blood flow, arterial lactate concentration and muscle metabolism.
2. Six male subjects performed intermittent arm exercise in two separate 32 min periods (Part I and Part II) and in one subsequent 20 min period in which one leg knee-extensor exercise was also performed (Part III). The exercise was performed at various intensities in order to obtain different steady-state arterial blood lactate concentrations. In the inactive leg, femoral venous blood flow (draining about 7·7 kg of muscles) was measured and femoral arterial and venous blood was collected frequently. Biopsies were taken from m. vastus lateralis of the inactive leg at rest and 10 and 30 min into both Part I and Part II as well as 10 min into recovery from Part II.
3. The arterial plasma lactate concentrations were 7, 9 and 16 mmol l^{-1} after 10 min of Parts I, II and III, respectively, and the corresponding arterial–venous difference ($a{-}v_{diff}$) for lactate in the resting leg was 1·3, 1·4 and 2·0 mmol l^{-1}. The muscle lactate concentration was 2·8 mmol (kg wet wt)$^{-1}$ after 10 min of Part I and remained constant throughout the experiment. During Parts I and II, $a{-}v_{diff}$ lactate decreased although the arterial lactate concentration and plasma–muscle lactate gradient were unaltered throughout each period. Thus, membrane transport of lactate decreased during each period.
4. Blood flow in the inactive leg was about 2-fold higher during arm exercise compared to the rest periods, resulting in a 2-fold higher lactate uptake. Thus, lactate uptake by inactive muscles was closely related to blood flow.
5. Throughout the experiment $a{-}v_{diff}$ for actual base excess and for lactate were of similar magnitude. Thus, in inactive muscles lactate uptake appears to be coupled to the transport of H^+.

A considerable portion of the lactate produced by intensely contracting muscles is released into the blood and some is extracted by inactive muscles (Bangsbo *et al.* 1990). Thus, inactive muscle may play an important role for lactate metabolism during and after exercise. Although several studies have focused on the uptake of lactate by inactive muscle, many unsolved issues remain. For instance, the importance of perfusion for the uptake of lactate by inactive muscles is not well established. Jorfeldt (1970) found that the lactate uptake by human forearm muscle at low lactate concentrations was related to the blood flow. On the other hand, in several studies using an isolated dog gastrocnemius–plantaris muscle preparation it was observed that the perfusion rate influenced the lactate exchange only to a minor extent (Hirche, Homback, Langohr, Wacker & Busse, 1975; Gladden & Yates, 1983; Gladden, Crawford & Webster, 1992).

The uptake of lactate by inactive muscles appears to be enhanced with increasing arterial blood lactate concentration (Ahlborg, Hagenfeldt & Wahren, 1975, 1976; Poortmans, Bossche & Lecaercq, 1978; Kowalchuk, Heigenhauser, Lindinger, Obminski, Sutton & Jones, 1988; Bangsbo, Gollnick, Graham & Saltin, 1991). However, it is unclear how lactate uptake changes during steady-state lactate concentrations and how it is related to metabolism within the inactive muscles. The extraction of lactate in inactive muscles may be accompanied by an uptake of protons (H^+), since the transport of lactate across the sarcolemma appears to be dominated by lactate–H^+ co-transport (Juel, 1988; Watt, MacLennan, Hundal, Kuret & Rennie, 1988; Juel & Wibrand, 1989; Watt, Gladden, Hundal & Crawford, 1994). Proton elimination from contracting muscles in man has been demonstrated to be faster than the lactate efflux (Bangsbo, Graham, Johansen & Saltin, 1993). However, little is known about the relationship between lactate and H^+ uptake in inactive muscles.

Thus, the aim of the present study was to examine how uptake of lactate by inactive muscle is associated with H^+ extraction and affected by blood flow, arterial lactate

concentration and muscle metabolism in man. Intermittent arm exercise was performed at various intensities to induce different steady-state arterial lactate concentrations and to obtain various blood flow rates to the inactive leg muscles. Measurements were performed over a resting leg.

METHODS

Subjects

Six healthy male subjects ranging in age from 22 to 29 years, with a mean height of 181 (range 171–181) cm, and a mean weight of 77·0 (65·0–83·3) kg participated in the experiment. The maximum oxygen uptake ($\dot{V}_{O_2,max}$) of the subjects was 54·3 (47·2–65·7) ml $min^{-1} kg^{-1}$. The subjects were fully informed of any risks and discomforts associated with these experiments before giving their informed consent to participate. The study was approved by the Copenhagen Ethics Committee.

Procedures

The subjects sat in an upright position and performed intermittent arm cranking or knee extensor exercise with the right leg, while the left (inactive) leg was in a horizontal position supported by straps. All subjects practised the exercise several times on separate days and at the final pre-experiment the entire protocol, except the invasive measurements, was performed.

About 3 h before the experiment the subjects had a light breakfast consisting of two slices of bread with marmalade and 500 ml juice, and reported to the laboratory 2 h prior to the experiment. After 30 min of rest in the supine position a catheter was placed in the femoral artery of the left leg under local anaesthesia with 2–3 ml lidocain (10 mg ml^{-1}) using a 20G cannula, and the tip was positioned 1–2 cm proximal to the inguinal ligament. A catheter was also placed in the femoral vein of the left (inactive) leg using local anaesthesia and an 18G needle. The tip of the catheter was positioned about 1–2 cm distal to the inguinal ligament. The thermistor for measurement of venous blood temperature was inserted through the catheter and was advanced 8–10 cm proximal to the tip.

Placement of the catheters was followed by 1 h of rest in the supine position. The subject then performed intermittent arm exercise alternating between 1 min of exercise at a work rate of 83 W and 1 min of rest for a total of 32 min (Fig. 1, Part I). This was immediately followed by another 32 min period with the same exercise pattern, but with a work rate of 108 W (Fig. 1, Part II). After 14 min of rest, the subject carried out Part III, which consisted of two identical exercise periods (Fig. 1). In each period the subject performed intense knee-extensor exercise with the right leg at a mean power output of 65 W (frequency, 60 kicks min^{-1}) to exhaustion (2–4 min) followed by four intense 1 min arm exercise bouts (128 W) separated by 30 s rest periods. Thus, in Part III lactate release was achieved from both the right leg and the arms.

During the entire experiment the left (inactive) leg was in a horizontal position and supported by straps, which allowed the leg to relax while the upper body and the right leg exercised. In order to determine if there was any activity in the muscles of the resting leg, surface electromyographic (EMG) activity of the quadriceps muscle of the inactive leg was recorded during the experiment.

Leg blood flow was determined and blood was then drawn simultaneously from the femoral artery and vein prior to and regularly during Parts I, II and III as shown in Fig. 1. In the samples taken during the arm exercise periods, and after 2 and 10 min of recovery from Parts II and III, only blood and plasma lactate was measured. Furthermore, leg blood flow was not determined during the arm exercise periods in Part III. An occlusion cuff placed just below the knee of the left leg was inflated (220 mmHg) prior to and during the entire experiment except for short breaks (10 s) and the last 3 min before Part III. A muscle biopsy was taken from m. vastus lateralis of the inactive leg prior to Part I, after 10 and 30 min of both Part I and II, and a further 10 min after Part II (Fig. 1).

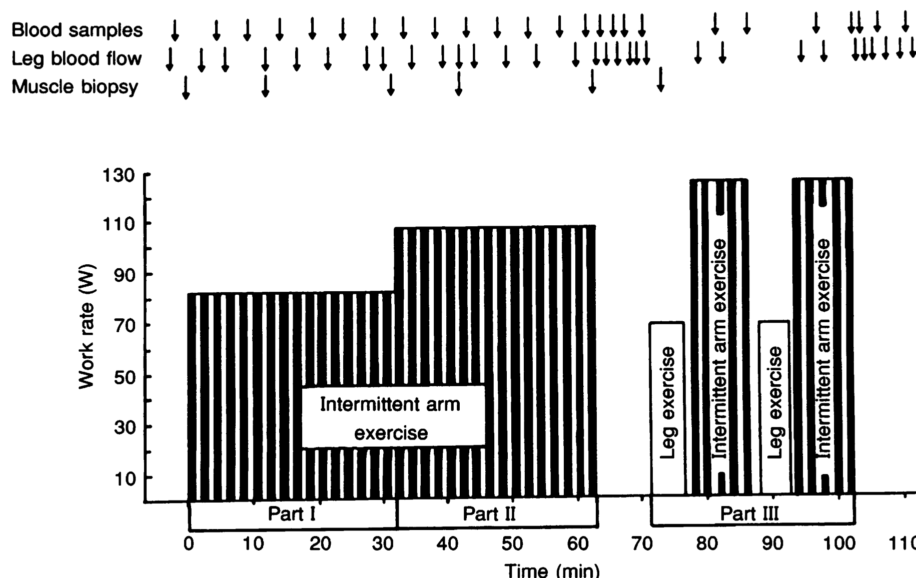

Figure 1

Schematic representation of the experimental design. ■, exercise; □, rest.

Blood flow and blood pressure. Femoral venous blood flow was measured by the thermodilution technique (Andersen & Saltin, 1985) modified for resting conditions (Richter, Mikines, Galbo & Kiens, 1989). Blood pressure was measured continuously using a Statham transducer connected to the femoral artery catheter.

Blood analysis. Blood oxygen saturation was determined spectrophotometrically (Radiometer OSM-2 Hemoximeter). Haemoglobin (Hb) concentration was determined with the Haemoximeter which was calibrated spectrophotometrically by the cyanomethemoglobin method (Drabkin & Austin, 1935). Haemoglobin concentrations at low oxygen saturation were adjusted with a correction factor obtained from multiple measurements of oxygen content of partly oxygenated blood samples as determined by Van Slyke analysis (Holmgren & Pernow, 1959). Haematocrit (Hct) determinations were made in triplicate, using microcentrifugation. P_{O_2}, P_{CO_2} and pH were measured with the Astrup technique, and from these, plasma HCO_3^- and actual base excess (ABE) were calculated as described by Siggaard-Andersen (1974; ABL 30, Radiometer, Copenhagen, Denmark). Since reduced Hb has a higher buffer capacity than fully oxygenated Hb, in each blood sample ABE was adjusted (ABE_{adj}) to fully oxygenated Hb (Siggaard-Andersen, 1974). Whole blood CO_2 content was determined from blood Hb, temperature, oxygen saturation, pH and P_{CO_2} according to the calculation described by Douglas, Jones & Reed (1988). A part of each blood sample was centrifuged rapidly, and the plasma was collected. Plasma and blood lactate were analysed from PCA-precipitated extractions of the blood samples, using a fluorometric assay (Lowry & Passonneau, 1972).

Muscle biopsies. Muscle samples were analysed for total water by weighing the samples before and after freeze drying, and for lactate, pyruvate and CP by fluorometric assays (Lowry & Passonneau, 1972).

Calculations

For each individual, leg blood flow during the arm exercise and the rest periods within each part of the experiment (Part I, II, and III) was determined as the average of the measurements obtained during exercise and rest, respectively. This appears valid as only small variations in leg blood flow were found within either rest or exercise periods (maximum $0{\cdot}4\ l\ min^{-1}$) and no systematic differences were observed. In recovery from Parts II and III a continuous blood flow curve was constructed for each subject by linear connection of the consecutive data points to obtain time-matched values for the blood flow measurements with the blood variables.

The red blood cell (RBC) lactate concentration was calculated with the following equation (Buono & Yeager, 1986):

$$Lactate_{RBC} = (lactate_{blood} - lactate_{plasma}(1 - Hct))\ Hct^{-1},$$

where Hct is $0{\cdot}98 \times$ measured Hct and where 0·98 corrects measured hematocrit for trapped plasma.

Net lactate uptake, O_2 uptake ($\dot{V}_{O_2}$) and CO_2 release ($\dot{V}_{CO_2}$) by the inactive muscles were calculated by multiplying the blood flow by the difference between femoral artery and venous ($a{-}v_{diff}$) concentrations of the variable.

The muscle lactate gradient was defined as the difference between the concentration of lactate in the femoral venous plasma water and the cell water, the former being the best estimate of the interstitial concentration. The intracellular lactate concentration was calculated from the measured concentration in the muscle sample and the water content based on the assumptions that cellular water was 85% of the total water (Sjögaard, Adams & Saltin, 1985).

The mass of the muscles perfused in the inactive leg ($7{\cdot}7 \pm 0{\cdot}4$ kg; $\pm$ S.E.M.) was estimated as the product of the ratio between the total mass of muscles perfused by the femoral artery (except the

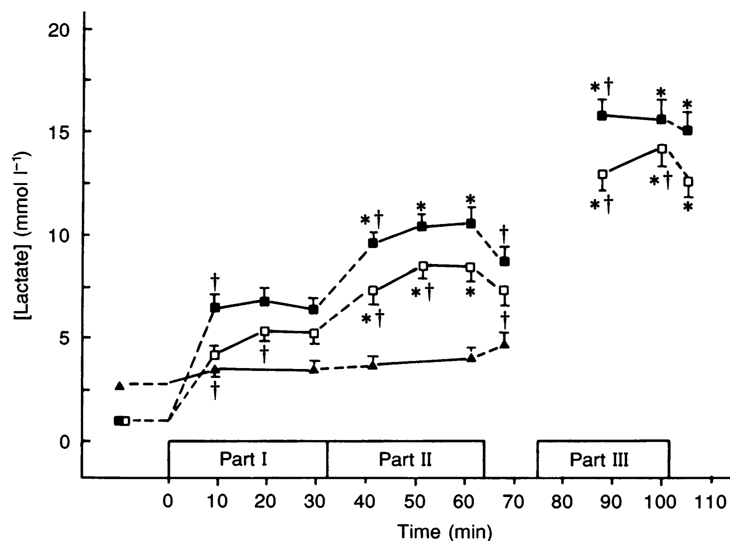

Figure 2. Plasma lactate concentration in arterial (■) and inactive leg femoral venous (□) blood as well as muscle intracellular lactate concentrations (▲) at rest and during rest periods in Parts I, II and III as well as after Parts II and III

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0{\cdot}05$) from the corresponding value in the previous part; † significantly different ($P < 0{\cdot}05$) from the preceding value. Values are means ± S.E.M.

muscles of the lower leg which were occluded during the measurements) and the mass of the quadriceps muscle (2·4 : 1; Bischoff *et al.* 1863) and the mass of the quadriceps muscle (3·24 ± 0·17 kg) of the present subjects. The latter was estimated based on Simpson's rule, which included measurements of thigh length, multiple circumferences of the thigh and the skinfold thickness (Jones & Pearson, 1969; Andersen & Saltin, 1985).

Statistics

Two-way analysis of variance (ANOVA) with repeated measures was used for comparison between each part of the experiment (Part I, II and III), and between values obtained during the exercise and the interspersed rest periods. If a significant F-value was observed then Students paired t test was used to locate the differences. A significance level of 0·05 was chosen. Standard error of the mean values (± S.E.M.) are only given in the text where this value cannot be obtained from a figure or a table.

RESULTS

Mean arterial pressure and leg blood flow

The mean arterial pressure (MAP) was 107 ± 6 mmHg at rest. During the intermittent arm exercise MAP was higher ($P < 0·05$), being 118 ± 3 (Part I), 127 ± 5 (Part II) and 126 ± 10 (Part III) mmHg during the rest periods, and 136 ± 2 (Part I) and 141 ± 5 (Part II) mmHg during the exercise periods.

The leg blood flow at rest was 0·32 ± 0·06 l min^{-1} or about 42 ml min^{-1} kg^{-1} muscle, and 0·53 ± 0·12 and 0·68 ± 0·14 l min^{-1} during the rest periods in Part I and Part II, respectively. These values were lower ($P < 0·05$) than during the exercise periods in Part I (1·11 ± 0·34 l min^{-1}) and Part II (1·24 ± 0·31 l min^{-1}). Leg blood flow was 0·89 ± 0·21 l min^{-1} during the rest periods in Part III.

After 2, 6, and 10 min of recovery from Part II, MAP was 110 ± 3, 107 ± 4, and 101 ± 5 mmHg, respectively, and the corresponding values after Part III were 116 ± 4, 105 ± 2, and 100 ± 4 mmHg, respectively. Leg blood flow at 2, 6, and 10 min was 0·45 ± 0·10, 0·38 ± 0·09 and 0·34 ± 0·06 l min^{-1} after Part II, respectively, and 0·54 ± 0·09, 0·36 ± 0·06 and 0·32 ± 0·05 l min^{-1} after Part III.

Lactate exchange

Arterial plasma lactate concentration increased from 1·3 to about 7, 9 and 16 mmol l^{-1} during the early phase of Part I, Part II and Part III, respectively, and remained constant within each period (Fig. 2). The corresponding arterial blood lactate concentration was about 4, 6 and 10 mmol l^{-1}.

Prior to Part I no net exchange of lactate was observed for the inactive leg. After 10 min of Part I, the $a-v_{diff}$ lactate was 1·3 mmol l^{-1}, which was similar to the corresponding value in Part II (1·4 mmol l^{-1}), but lower ($P < 0·05$) than during Part III (1·9 mmol l^{-1}; Fig. 3). During Parts I and II, $a-v_{diff}$ lactate decreased ($P < 0·05$) with time to 0·8 and 1·1 mmol l^{-1}, respectively. The net lactate uptake followed the same pattern (Fig. 3). No difference in $a-v_{diff}$ lactate within Part I and Part II was observed whether the arms were exercising or resting. Thus, as a result of the elevated blood flow, the net lactate uptake was higher ($P < 0·05$) during the arm exercise periods compared to the rest periods in both Parts I and II (Fig. 3). In recovery from Parts II and III the $a-v_{diff}$ lactate decreased ($P < 0·05$), but it was still positive ($P < 0·05$) after 10 min (Fig. 3). Throughout the experiment the $a-v_{diff}$ lactate for red blood cells was never different from nil (Fig. 4).

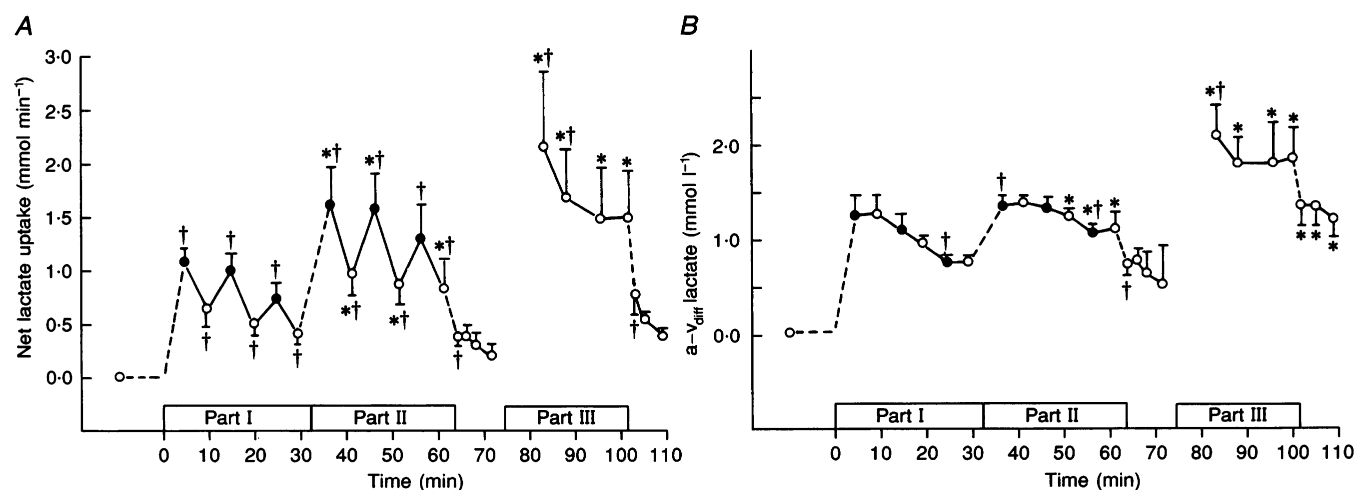

Figure 3. Net lactate uptake (*A*) and $a-v_{diff}$ lactate (*B*) across the inactive leg at rest as well as during exercise (●) and rest (○) periods in Parts I, II and III, and also after Parts II and III

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0·05$) from the corresponding value in the previous part; † significantly different ($P < 0·05$) from the preceding value. Values are means ± S.E.M.

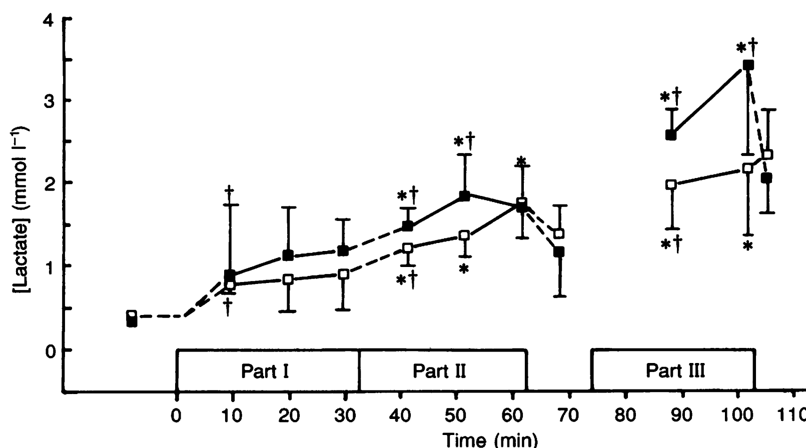

Figure 4. Red blood cell lactate concentration in arterial (■) and inactive leg femoral venous (□) blood at rest and during rest periods in Parts I, II and III as well as after Parts II and III

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0{\cdot}05$) from the corresponding value in the previous part; † significantly different ($P < 0{\cdot}05$) from the preceding value. Values are means ± S.E.M.

The muscle lactate concentration increased ($P < 0{\cdot}05$) from 1·9 to 2·8 mmol (kg wet wt)$^{-1}$ during the first 10 min of Part I, and thereafter it remained constant (Table 1). The muscle lactate gradient (femoral venous plasma lactate concentration − intracellular lactate concentration) was higher ($P < 0{\cdot}05$) during Part II compared to Part I (Fig. 2). The net uptake of lactate was related to both the arterial lactate concentration and the muscle lactate gradient, but the net uptake was lowered at a given arterial lactate concentration and muscle lactate gradient as a function of time (Fig. 5).

Muscle pyruvate of 0·06 mmol (kg wet wt)$^{-1}$ at rest was unchanged throughout the experiment (Table 1). The ratio between muscle lactate and pyruvate increased ($P < 0{\cdot}05$) from 36·4 at rest to 56·9 and 52·0 after 30 min of Part I and Part II, respectively. The ratio 10 min after Part II was not different from rest.

Acid–base balance

Arterial and femoral venous blood pH decreased during Part I and further during Part II, with no difference in pH between arterial and venous values (Fig. 6). There was a

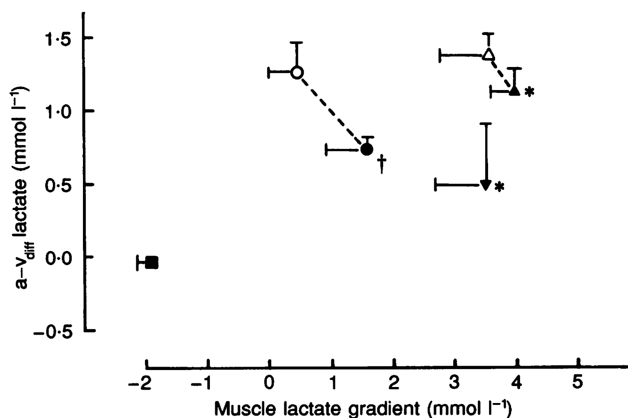

Figure 5. Muscle lactate gradient (intracellular muscle lactate–venous plasma lactate concentration) related to a–v$_{diff}$ lactate across the inactive leg

Values are rest (■), 10 min (○,△) and 30 min (●,▲) of Part I (○,●) and Part II (△,▲), as well as recovery from Part II (▼). * Significantly different ($P < 0{\cdot}05$) from the 10 min of Part II; † significantly different ($P < 0{\cdot}05$) from 10 min of Part I. Values are means ± S.E.M.

Table 1. Muscle CP, lactate and pyruvate (mmol (kg wet wt)$^{-1}$) as well as % H_2O during intermittent arm exercise (Part I and Part II) and after 10 min of recovery (Rec)

		Part I		Part II		Rec
Time (min)	0	10	30	40	60	70
CP	20·9 ± 0·5	19·1 ± 0.7	19·4 ± 0·5	18·5 ± 0·9	19·8 ± 0·9	19·5 ± 0·8
Lactate	1·91 ± 0·25	2·80 ± 0·26*	2·80 ± 0·23*	3·37 ± 0·23*	3·54 ± 0·32*	3·38 ± 0·35*
Pyruvate	0·06 ± 0·02	0·08 ± 0·01	0·06 ± 0·01	0·06 ± 0·01	0·07 ± 0·01	0·10 ± 0·02 *
% H_2O	74·8 ± 0·2	75·6 ± 0·7	75·3 ± 0·2	76·4 ± 0·7	75·3 ± 0·3	75·3 ± 0·4

Values are given as means ± S.E.M. * Significantly different ($P < 0·05$) from 0 min (rest).

continued decline in pH in Part III, and venous pH was lower ($P < 0·05$) than arterial pH. Both arterial and venous ABE_{adj} of 0·2 mmol l^{-1} at rest decreased during Part I and Part II, but the arterial concentration more than the venous concentration (Fig. 6). Thus, throughout the experiment $v-a_{diff}$ ABE_{adj} was positive. There was a correlation between the $v-a_{diff}$ of ABE_{adj} and lactate ($r = 0·90$; $P < 0·05$) and the differences between these variables were not different from nil, except after 10 min of Part I (−0·26 ± 0·06 mmol l^{-1}; $P < 0·05$) and in recovery from Part III (0·82 ± 0·24 mmol l^{-1}; $P < 0·05$).

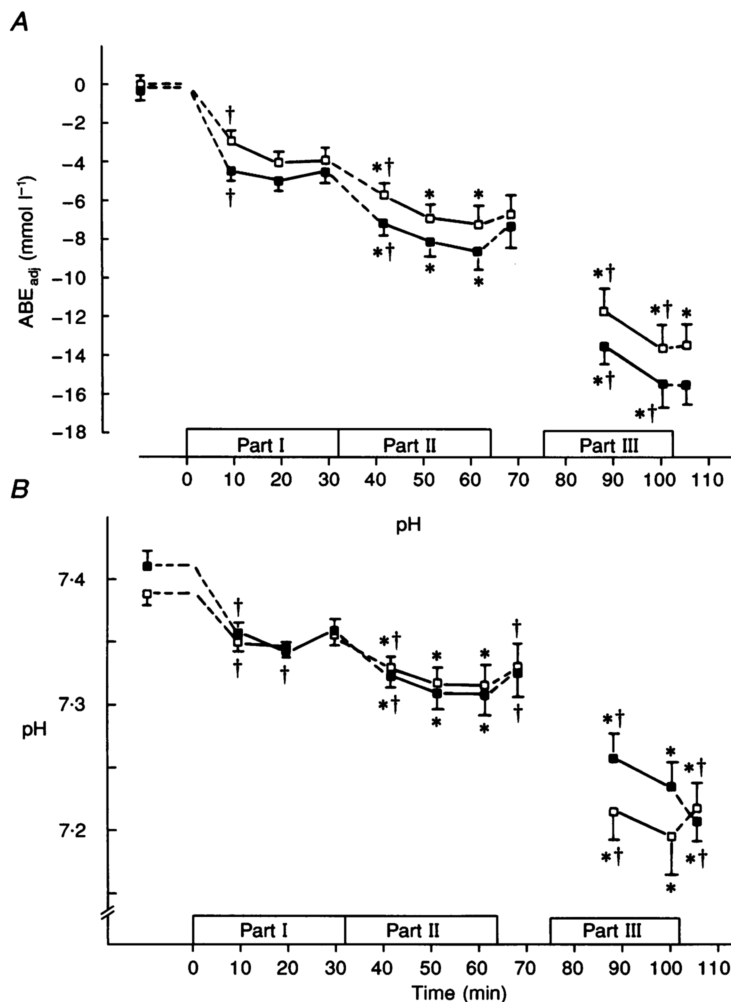

Figure 6. ABE_{adj} (*A*) and pH (*B*) in arterial (■) and inactive leg femoral venous (□) blood at rest and during rest periods of Parts I, II and III as well as after Parts II and III.

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0·05$) from the corresponding value in the previous part; † significantly different ($P < 0·05$) from the preceding value. Values are means ± S.E.M.

Both arterial and femoral venous HCO_3^- decreased throughout the experiment (Fig. 7). During Part I and Part II the v–a_{diff} HCO_3^- was 2–3 mmol l^{-1} and lower ($P < 0.05$) than in Part III (about 6 mmol l^{-1}). The venous P_{CO_2} was 44·2 mmHg at rest and it became lower ($P < 0.05$) during Part I and Part II, but during Part III it was higher ($P < 0.05$) than at rest (Fig. 7). Arterial P_{CO_2} decreased throughout the experiment.

Leg $\dot{V}_{O_2}$ increased ($P < 0.05$) from 19 ml min^{-1} at rest to 34 ml min^{-1} after 10 min of Part I, and it increased ($P < 0.05$) further during Part II being 47 ml min^{-1} after 10 min (Fig. 8). During Part III leg $\dot{V}_{O_2}$ was higher ($P < 0.05$) than during Part II. Leg $\dot{V}_{CO_2}$ values in the early phase of Part I (39 ml min^{-1}), during Part II (43–44 ml min^{-1}) and Part III (100–120 ml min^{-1}) were higher ($P < 0.05$) than at rest (17 ml min^{-1}; Fig. 8). The ratio between leg $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ was 1·47 and 0·95 after 10 and 30 min of Part I, and the corresponding values for Part II were 1·19 and 0·92, respectively. It was 1·2–1·3 during Part III.

DISCUSSION

The major findings of the present study were that the uptake of lactate in inactive muscles was closely related to blood flow and that it decreased as a function of time, although the arterial plasma lactate concentration and muscle lactate gradient were unaltered. Furthermore, the uptake of lactate and H^+ by inactive muscles was of equal magnitude.

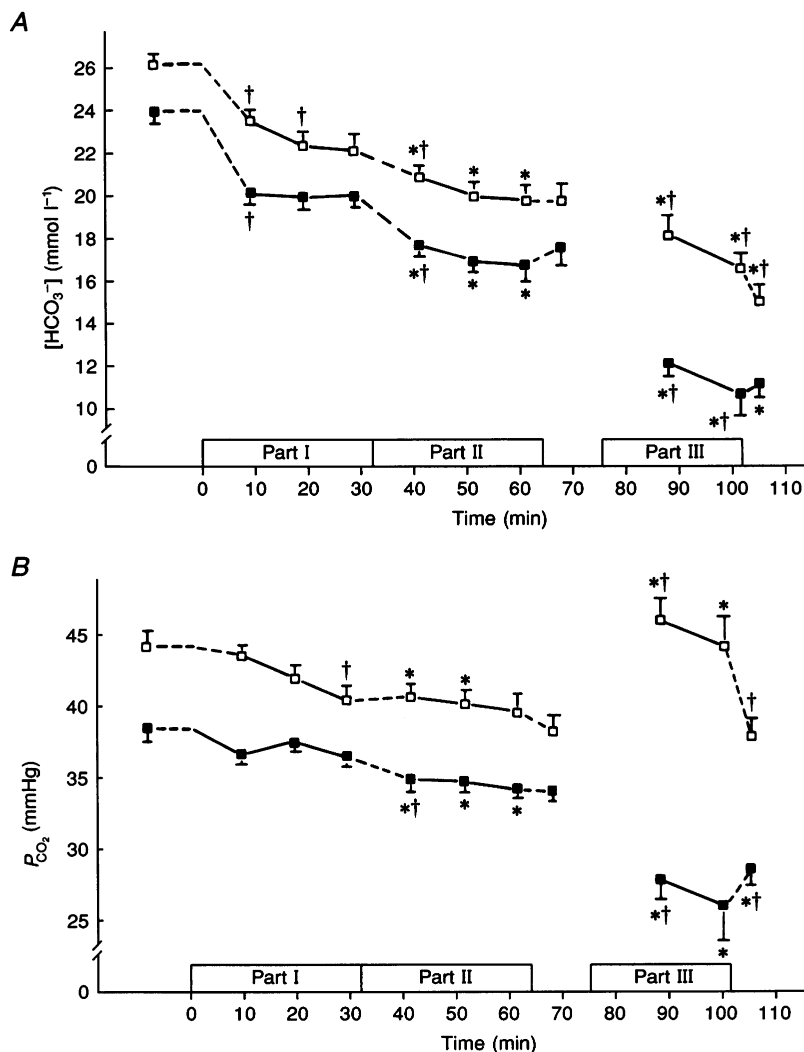

Figure 7. $[HCO_3^-]$ (*A*) and P_{CO_2} (*B*) in arterial (■) and inactive leg femoral venous (□) blood at rest and during rest periods of Parts I, II and III as well as after Parts II and III.

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0.05$) from the corresponding value in the previous part; † significantly different ($P < 0.05$) from the preceding value. Values are means ± S.E.M.

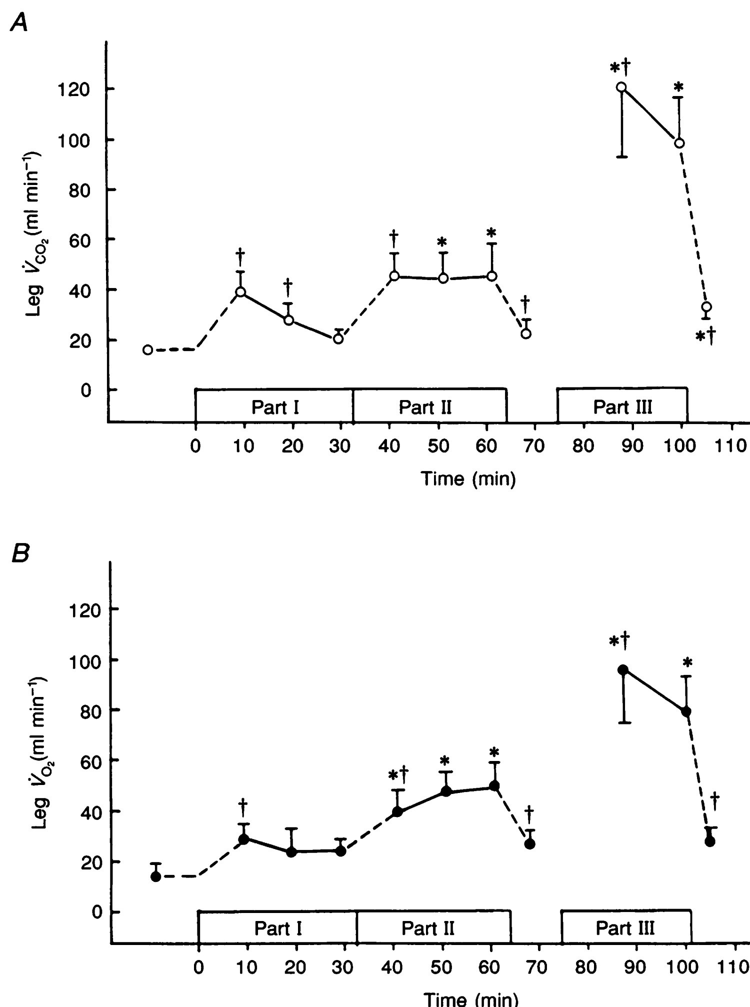

Figure 8. Leg $\dot{V}_{CO_2}$ (*A*) and $\dot{V}_{O_2}$ (*B*) for the inactive leg at rest and during rest periods of Parts I, II and III as well as after Parts II and III

A dashed line indicates a shift between two conditions. * Significantly different ($P < 0.05$) from the corresponding value in the previous part; † significantly different ($P < 0.05$) from the preceding value. Values are means ± S.E.M.

An important consideration is whether the muscles of the inactive leg were completely inactive during exercise involving the other limbs. During Part I and Part II no EMG activity was detected in the resting thigh muscles and no change in muscle CP concentration was observed, suggesting that there was no activity in the inactive leg during these phases of the experiment. During the exercise periods in Part III, a small amount of EMG activity (less than 3% of the activity during a maximal isometric contraction) was recorded in the resting leg, indicating a minor degree of activity in the muscles. However, these low intensity contractions are not likely to have influenced the results to any major extent.

Blood flow of the inactive leg was elevated during the exercise periods with the arms (1·1–1·2 l min^{-1} approximately equal to 140–160 ml min^{-1} kg^{-1}) compared to the rest periods in between (0·5–0·7 l min^{-1} approximately equal to 60–90 ml min^{-1} kg^{-1}). This made it possible to examine how blood flow to the inactive muscles affected the lactate uptake, as the arterial lactate concentration was the same during the exercise and rest periods. The lactate uptake rose in proportion to the increase in blood flow, since the a–v_{diff} lactate was the same at the two levels of blood flow. This is likely to be a result of a larger capillary surface area available for lactate transport when the blood flow is elevated (Connett, Gayeski & Honig, 1986).

In agreement with the finding in the present study Jorfeldt (1970) observed that the lactate uptake by inactive human forearm muscle at a low lactate concentration (3 mmol l^{-1}) was related to the delivery of lactate rather than the arterial lactate concentration. In addition, in a recent study Watt *et al.* (1994) observed that the tracer-measured lactate influx in the perfused rat hindlimb was related to the blood

flow in the range 140–600 ml min^{-1} kg^{-1} at a low lactate concentration in the perfusate. In contrast, Gladden & Yates (1983) found that lactate uptake was unaltered in resting dog muscles, despite a considerable variation in blood flow (range, 90–180 ml min^{-1} kg^{-1}) and thereby lactate delivery to the muscle. However, the distribution of muscle blood flow is likely to be different in the *in vitro* compared to the present *in vivo* condition, which may explain the discrepancy between the human studies and the dog study (Honig, Odoroff & Frierson, 1982).

The $a-v_{diff}$ lactate across the inactive muscles was related to the arterial blood lactate concentration in accordance with other human studies (Ahlborg *et al.* 1975, 1976; Poortmans *et al.* 1978; Stanley *et al.* 1986; Kowalchuk *et al.* 1988; Bangsbo *et al.* 1991). However, the uptake of lactate decreased progressively during Part I and the last phase of Part II even though the arterial lactate concentration and muscle lactate gradient were unaltered. Similar findings were obtained in studies perfusing the rat hindlimb (Gladden & Yates, 1983; Gladden, 1989; Chin, Lindinger & Heigenhauser, 1991) and in a human study (Ahlborg *et al.* 1975). In the latter study $a-v_{diff}$ lactate decreased from 2·0 mmol l^{-1} after 5 min to 0·6 mmol l^{-1} after 15 min with an unaltered arterial lactate concentration of 4 mmol l^{-1}. The high $a-v_{diff}$ lactate at the initial phase of Part I most likely reflects a fast diffusion of lactate into the interstitial space as a result of the changes in arterial lactate concentration. On the other hand, it is likely that lactate equilibrium was reached soon after the change from Part I to Part II, as the ratio between intracellular and plasma venous lactate was similar after 10 and 30 min (0·54 and 0·49, respectively) of Part II. Therefore, the decrease in the last phase of Part II seems to reflect a decline in the net transport of lactate across the muscle sarcolemma. This decrease in membrane transport capacity may have been caused by muscle acidification, as the uptake of lactate appeared to be tightly coupled to an equal uptake of H^+ probably due to the action of the lactate–H^+ cotransporter demonstrated in *in vitro* studies (Koch, Webster & Lowell, 1981; Juel, 1988; Watt *et al.* 1988; Juel & Wibrand, 1989; Watt *et al.* 1994). In agreement with this suggestion, Mason & Thomas (1988) observed an intracellular acidification, when perfusing the frog sartorius muscle with solutions containing lactate in the range 5–60 mmol l^{-1}. Although the buffer capacity of muscle is high (Juel, Bangsbo, Graham & Saltin, 1990), the uptake of H^+ in the present study may have lead to decreased muscle pH which in turn decreased lactate transport (Juel & Wibrand, 1989). In addition, a greater muscle acidification would have elevated the ratio between undissociated lactic acid and lactate ions in the muscle, which may have decreased the uptake of lactate by free diffusion. Thus, it is possible that a decrease in muscle pH was the cause of the progressive reduction in lactate uptake in the inactive muscles at a constant arterial lactate concentration.

In the present study no significant $a-v_{diff}$ lactate for red blood cells was observed, suggesting that the lactate taken up by the inactive muscles is only to a very minor extent released from the red blood cells during passage of the muscles. This is in accordance with the finding in humans of a slow rate of lactate equilibrium between red blood cells and plasma (Juel *et al.* 1990). In contrast, Lindinger, Heigenhauser, McKelvie & Jones (1990) and McKelvie, Lindinger, Jones & Heigenhauser (1992) found pronounced differences between arterial and venous red blood cell lactate concentration in inactive muscles, and they suggested that the major part of the lactate extracted by the inactive muscles was released from the red blood cells. The discrepancy between these studies and the present study may be related to differences in the procedures used to separate the plasma from the red blood cells. Lindinger *et al.* (1990) and McKelvie *et al.* (1992) centrifuged the blood at a speed of 5000 r.p.m. (*g* not reported) for 15 min, while in the present study the blood was centrifuged at a speed of 20000 r.p.m. (18000 *g*) for 1 min. The low speed and long centrifugation time in the studies by Lindinger *et al.* (1990) and McKelvie *et al.* (1992) may have allowed a significant exchange of lactate between plasma and the red blood cells, which leads to an overestimation of the release of lactate over the red cell membrane through passage of the capillaries. This problem was minimized in the present study and it is likely that the plasma and erythrocyte lactate levels measured in the present study better reflect the *in vivo* condition. It thus appears that the red blood cells do not contribute significantly to the release of lactate to the inactive muscles.

Most of the lactate taken up by the inactive muscles was metabolized, since after an initial accumulation no further increase in muscle lactate occurred. Gutierrez *et al.* (1994) observed that lactate uptake in the rabbit hindlimb was reduced when the cytosolic malic reaction was blocked by hydroxymalonate. Thus, part of the lactate may have been converted to malate. Another part of the lactate was probably oxidized. The oxygen uptake of the inactive muscles was elevated during the intermittent arm exercise. This may have been due to a higher cytosolic NADH concentration secondary to lactate uptake, in turn leading to an increased mitochondrial uptake of NADH, which may have stimulated a higher rate of respiration. In agreement with this notion is the observation of an elevated muscle lactate/pyruvate ratio, reflecting an increased $NADH_2$/NAD ratio within the muscles and perhaps a change in the LDH activity. Similarly, Chin *et al.* (1991) found an increased lactate/pyruvate ratio and oxygen uptake when the rat hindlimb was perfused with a perfusate containing lactate at a concentration of 10 mmol l^{-1}. In accordance with the suggestion that lactate was oxidized, Gladden, Crawford & Webster (1994) found that the lactate uptake by canine skeletal muscle increased when the metabolic rate of the muscle was increased.

The CO_2 release from the inactive muscles was also elevated when the arterial lactate concentration was increased. The marked release of CO_2 may explain the fact that no $a\text{-}v_{diff}$ pH was observed during Part I and II, and that a positive difference was found in Part III, despite a considerable uptake of H^+. The ratio between leg $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ was higher than 1 in the early phase of Part I and Part II, and throughout Part III, which suggests that CO_2 was released in excess of that produced through oxidation. Similar observations were made in a study perfusing the rat hindlimb with a perfusate containing high lactate concentrations (Chin *et al.* 1991). Part of the CO_2 may have been derived from HCO_3^- buffering of H^+. Another part may have been released from muscle proteins through a change in muscle acidification, as it is possible that an increase in muscle H^+ caused release of CO_2 from amino groups. This suggestion is supported by the finding of a more pronounced release of CO_2 during Part III, when the largest change in muscle pH would be expected as a result of the elevated proton uptake.

In summary, the present study demonstrates that in humans lactate uptake by inactive muscles is related to blood flow and is furthermore coupled to the transport of H^+. The ensuing acidification of muscle may be responsible for decreased sarcolemmal lactate transport with time.

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