Journal of Physiology (1993), **462**, *pp.* 115–133
With 7 figures
Printed in Great Britain

LACTATE AND H^+ EFFLUXES FROM HUMAN SKELETAL MUSCLES DURING INTENSE, DYNAMIC EXERCISE

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(*Received 13 March 1992*)

SUMMARY

1. Lactate and H^+ efflux from skeletal muscles were studied with the one-legged knee extension model under conditions in which blood flow, arterial lactate and the muscle–blood lactate concentration gradient were altered. Subjects exercised one leg twice to exhaustion (EX1, EX2), separated by a 10 min recovery and a period of intense intermittent exercise. After 1 h of recovery the exercise protocol was repeated with the other leg. Low-intensity exercise was performed with one leg during the recovery periods, while the other leg was passive during its recovery periods.

2. Prior to, and immediately after, EX1 and EX2 and then 3 and 10 min after EX1, a biopsy was taken from the vastus lateralis of the exercised leg for lactate, pH, muscle water and fibre-type determinations. Measurements of leg blood flow and venous–arterial differences for lactate (whole blood and plasma), pH, partial pressure of CO_2 (P_{CO_2}), haemoglobin, saturation and base excess (BE) were performed at the end of exercise and regularly during the recovery period after EX1.

3. The lactate release was linearly related ($r = 0{\cdot}96$; $P < 0{\cdot}05$) to the muscle lactate gradient over a range of muscle lactate from 0 to 45 mmol (kg wet wt)$^{-1}$. The muscle lactate transport was evaluated from the net femoral venous–arterial differences ($V–A_{diff}$) for lactate. This rose with increases in the muscle lactate gradients, but as the gradient reached higher levels the $V–A_{diff}$ lactate responded less than at smaller gradients. Thus, the lactate transport over the muscle membrane appears to be partly saturated at high muscle lactate concentrations.

4. The percentage of slow twitch (% ST) fibres was inversely related to the muscle lactate gradient, but it was not correlated to the lactate release at the end of the exercises. In spite of a significantly higher blood flow during active recovery, the lactate release was the same whether the leg was resting or performed low-intensity exercise in the recovery periods. In several other conditions the muscle lactate and H^+ gradients would have predicted that the $V–A_{diff}$ lactate would have been greater than it actually was. Thus, a variety of factors affect muscle lactate transport, including arterial lactate concentration, muscle perfusion, muscle contraction pattern and muscle morphology.

5. The muscle and femoral venous pH declined during EX1 to 6·73 and 7·14–7·15,

respectively, and they increased to resting levels during 10 min of either passive or active recovery. The ratio between proton release and lactate efflux was estimated from the BE difference across the muscle, adjusted for changes in reduced haemoglobin. This was 1·4–1·6 at the end of each exercise and remained greater than unity during recovery. Thus, proton release appears to be faster than the lactate efflux both during exercise and recovery.

INTRODUCTION

During intense exercise, energy is provided from glycolysis in the muscles leading to formation of lactate, which is released by simple and facilitated diffusion (Juel, 1988; Watt, MacLennan, Hundal, Kuret & Rennie, 1988). It has been suggested that the upper limit for lactate efflux is 5 mmol min^{-1} (Jorfeldt, Juhlin-Dannfelt & Karlsson, 1978). However, in more recent studies higher effluxes have been obtained and no saturation was apparent (Katz, Broberg, Sahlin & Wahren, 1986; Juel, Bangsbo, Graham & Saltin, 1990).

The diffusion of lactate across the sarcolemma appears to be dominated by a lactate–H^+ co-transport mechanism (Juel, 1988; Watt *et al.* 1988; Juel, 1991), but the exact elimination kinetics of lactate and H^+ ions from muscle cells during contraction and subsequent recovery has been the subject of various studies with apparently contradictory results. Proton elimination from the muscles has been reported to be faster than the lactate efflux in rats (Heigenhauser, Lindinger & Spriet, 1985), in dog muscles (Chirtel, Barbee & Stainsby, 1984) and in human muscles (Medbø & Sejersted, 1985). However, a 1:1 ratio has also been suggested from studies of dog (Stainsby & Eitzman, 1988) and human (Katz, Sahlin & Juhlin-Danfeldt, 1985) muscles, whereas a ratio below one has been reported for frog muscles (Mainwood & Worsley-Brown, 1975). It has also been proposed from *in vitro* studies that the ratio is increased by enhancing blood flow (Heisler, 1988). In most of the human studies only changes in lactate concentrations and base deficit in either arterial or arterialized blood were determined, which limits the information, as the uptake rates of lactate and H^+ equivalents by non-exercised tissue might have been unequal. A more direct evaluation can be performed by determining the venous–arterial difference over the exercising muscles.

Lactate continues to be released during recovery from exercise, and only a small portion of the lactate accumulated after exercise is metabolized in the muscle (Bangsbo, Gollnick, Graham & Saltin, 1991). It is well established that low-intensity exercise accelerates lactate removal from the blood (Hermansen & Stensvold, 1972). However, the influence of such exercise, and the accompanying elevated blood flow to the active muscles, on lactate and H^+ release from the muscles is not well established, and knowledge about the effect of elevated muscle and blood lactate prior to intense exercise on the flux of these variables during exercise is also lacking.

Thus, the aim of the present study was to examine to what extent blood flow, arterial lactate concentration and muscle morphology influence muscle lactate and H^+ exchange in an isolated small muscle group. In order to generate variation in these variables, repeated intense exercise bouts were performed, separated by either rest or very light contractions. The muscle contractions were confined to the knee extensors of one leg to allow for precise quantification of net uptakes and releases.

METHODS

Subjects

Six healthy male subjects ranging in age from 22 to 26 years, with an average height of 182 (range, 174–191) cm, and a mean weight of 75 (63–86) kg participated in the experiment. All subjects were habitually physically active, but none trained for competition. The mean fibre distribution of the quadriceps muscles for these subjects was 69·6 (21·8–79·3) % slow twitch (ST) fibres, 27·8 (9·6–46·4) % fast twitch a (FTa) fibres and 12·6 (3·1–31·8) % fast twitch b (FTb) fibres. The subjects were fully informed of any risks and discomfort associated with these experiments before giving their consent to participate. The study was approved by the local ethics committee.

Procedures

Subjects performed one-legged exercise in the supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscles (Andersen, Adams, Sjøgaard, Thorboe & Saltin, 1985). All subjects practised the exercise with each leg on more than four separate occasions before the final experiment was performed. At the final pre-experiment the subjects completed the entire protocol for the experiment (see below) except for the invasive measurements. About 1·5 h before the actual experiment a catheter was placed in the femoral artery using the Seldinger technique with the tip placed 1–2 cm proximal to the inguinal ligament. Two catheters were put in each of the two femoral veins. The tip of one of the catheters was positioned approximately 8 cm in the retrograde direction, i.e. 10–12 cm distal to the inguinal ligament. This catheter was used for collecting blood samples and for infusing the ice-cold saline. Another venous catheter was placed in the inguinal region with the tip 1–2 cm distal to the ligament. The thermistor for measurement of venous blood temperature was inserted through this catheter and was advanced just proximal to the tip.

Protocol

On the morning of the experiment, subjects arrived after a light breakfast. Placement of catheters was followed by 30 min of rest in the supine position. Then, the subjects warmed up one of the legs (randomly selected) for 10 min at a work rate of 10 W. After at least 10 min rest the same leg performed an intense power output ($59{\cdot}0 \pm 5{\cdot}3$ W with a kick frequency (f) of 60 min^{-1}) to exhaustion (EX1). This was followed by a 10 min period with either rest (passive leg, P-leg) or low-intensity exercise at a work rate of 10 W (active leg, A-leg). The recovery mode was randomly assigned. Then, the subject exercised the leg with seven bouts of intermittent exercise: high-intensity exercise ($90{\cdot}0 \pm 4{\cdot}5$ W; $f = 80$) for 15 s followed by either rest (P-leg) or low-intensity exercise (13 W; A-leg) for 15 s, in total 3·5 min. The recovery mode during this phase was selected to match that assigned in the first recovery period. The last of the seven intense exercise bouts was followed by a 2·5 min period with either rest or low-intensity exercise (10 W) again assigned to match that of the first recovery period. Then, the exhaustive exercise was repeated (EX2) with the same work rate as during EX1. After 1 h of rest (during which time no measurements were made) the other leg performed the same protocol, but with the opposite activity (i.e. active *vs.* passive) during the recovery periods between EX1 and EX2.

During the warm up, at rest before and at the end of the intense exercises, and furthermore, in the 10 min recovery from EX1, blood flow was determined frequently followed by blood sampling from the femoral artery and vein. Complete measurements were accomplished after about 0·8, 1·7, 3, 5·5, 8 and 10 min of recovery. An occlusion cuff placed just below the knee was inflated (220 mmHg) during measurements before and during the entire period of the intense exercise bouts and recovery periods. Before and immediately after the exhaustive exercise bouts a muscle biopsy was taken from m. vastus lateralis. Additional muscle biopsies were taken after 3 and 10 min of recovery both for the P- and A-leg. Figure 1 summarizes the protocol, including sampling times.

Blood flow. Femoral venous blood flow was measured by the thermodilution technique (Andersen & Saltin, 1985). Briefly, ice-cold saline was infused at a constant rate (115 ml min^{-1}) into the femoral vein for 10–15 s to achieve a drop in blood temperature of approximately 1 °C. At rest and in late recovery, when the blood flow was low, a 30–45 s infusion period was used.

Blood analysis. Blood oxygen saturation was determined spectrophotometrically (Radiometer OSM-2 Hemoximeter, Copenhagen, Denmark). Haemoglobin (Hb) concentration was determined with the Hemoximeter which was calibrated spectrophotometrically by the cyanomaethemoglobin

method (Drabkin & Austin, 1935). Hb concentrations at low oxygen saturation were adjusted with a correction factor obtained from multiple measurements of oxygen content of fully oxygenated blood samples as determined by Van Slyke analysis (Holmgren & Pernow, 1959). Partial pressure of O_2 (P_{O_2}) and CO_2 (P_{CO_2}) and pH were measured with the Astrup technique and from these, plasma

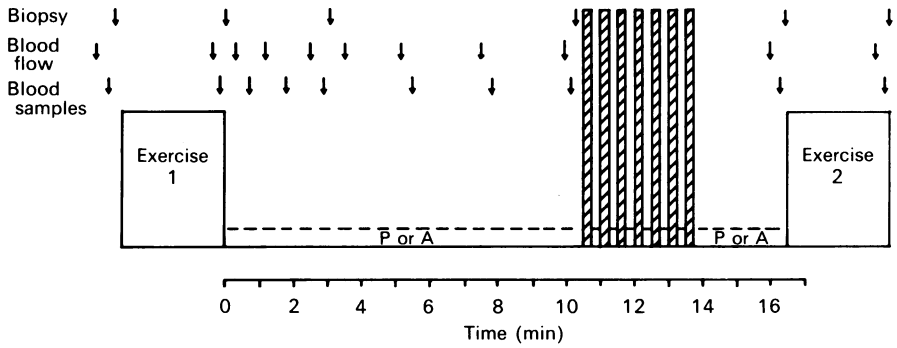

Fig. 1. Schematical representation of the experimental design. The hatched bars illustrate the seven 15 s intense exercise bouts (90 W) that were performed between EX1 and EX2. The dashed horizontal line indicates the low-intensity exercise during the recovery periods in the part of the experiment with 'active' recovery (A). In the other part of the experiment the leg was resting (P).

HCO_3^- and actual base excess (ABE) were calculated as described by Siggaard-Andersen (1974) (ABL 30, Radiometer, Copenhagen, Denmark). A part of each blood sample was centrifuged rapidly, and the plasma was collected. Lactate and glucose were analysed from PCA-precipitated extractions of the blood samples using a fluorometric assay (Lowry & Passonneau, 1972).

Muscle mass. The mass of quadriceps femoris muscles was estimated based on Simpson's rule, which included measurements of thigh length, multiple circumference of the thigh and the skin-fold thickness (Jones & Pearson, 1969). This anthropometric approach has given values similar to estimates based on multiple CAT scans (Saltin, 1985). The mean mass of the knee extensor for the A-leg and the P-leg was $3{\cdot}16 \pm 0{\cdot}15$ ($\pm$S.E.M.) and $3{\cdot}22 \pm 0{\cdot}18$ kg, respectively.

Muscle biopsies. Each muscle sample was analysed for total water by weighing the sample before and after freeze drying. Subsequently it was analysed for lactate by a fluorometric assay (Lowry & Passonneau, 1972). Muscle pH was measured by a small glass electrode (GK2801, Radiometer, Copenhagen, Denmark) after homogenizing the muscle sample in a non-buffering solution containing (mM): 145 KCl, 10 NaCl and 5 iodoacetic acid (Parkhouse, McKenzie, Hochachka & Ovalle, 1985). In addition, a piece of the biopsy taken before EX1 was frozen separately, and later used for serial cross-sectioning (10 μm) and stained for myofibrillar ATPase after alkaline and acid preincubation (Brooke & Kaiser, 1970) for identification of fibre types and determination of capillaries (Andersen, 1975).

Calculations

Lactate exchange and ABE change. Net lactate exchange by the thigh and the change in blood ABE across the thigh were calculated by multiplying the blood flow with the femoral venous–arterial difference ($V-A_{diff}$). The total net lactate exchange and net blood ABE change for the thigh during recovery from EX1 were calculated as described earlier (Bangsbo *et al.* 1991).

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

Statistics

Differences between values obtained for the A-leg and P-leg, differences between EX1 and EX2, and differences between values after 10 min of recovery from EX1 and values obtained during warming up, or at rest or zero were determined by the Wilcoxon ranking test for paired data (Pratt's modification, see Siegel, 1965). A significance level of 0·05 was chosen. Differences are not significant unless stated, and standard error of the mean value (±S.E.M.) is only given in the text where this value cannot be obtained from a figure or a table.

RESULTS

Leg blood flow

The leg blood flow of 4·11 (P-leg) and 4·66 (A-leg) l min^{-1} at the end of the EX1 bouts (duration, 3·7 and 3·4 min, respectively) decreased during the first 3 min of recovery to 2·55 and 3·43 l min^{-1} ($P < 0·05$), respectively. For the P-leg a further decline occurred during the next 7 min and the blood flow of 1·10 l min^{-1} after 10 min was lower ($P < 0·05$) than for the A-leg (2·92 l min^{-1}), but both values were higher ($P < 0·05$) than at rest (about 0·5 l min^{-1}). The blood flow prior to EX2 was also higher ($P < 0·05$) for the A-leg (3·2 l min^{-1}) than for the P-leg (2·4 l min^{-1}), but at the end of EX2 (duration, 3·0 min for both P- and A-leg) the flows were not different and they were similar to those of EX1.

Lactate release and muscle lactate gradient (Figs 2 and 3)

The femoral venous plasma lactate concentrations prior to the EX1 bouts were 0·8–1·0 mmol l^{-1} and they increased during EX1 to 10–11 mmol l^{-1} for either leg (Fig. 2). A further rise was observed during the first 0·8 min of recovery; thereafter it decreased to 10·3 (P-leg) and 9·2 (A-leg) mmol l^{-1} after 3 min. The latter two values were different from each other ($P < 0·05$), and the venous plasma lactate concentration remained higher ($P < 0·05$) for the P-leg compared to the A-leg. The venous plasma concentrations were elevated to 11·8 (P-leg) and 10·3 (A-leg) mmol l^{-1} before EX2 with no difference between the two legs, and no changes occurred during EX2. The arterial plasma lactate concentrations increased during EX1 to 6·3 mmol l^{-1} for either leg (Fig. 2). A further increase occurred during early recovery, whereafter it decreased slowly during the remaining part of recovery. The arterial plasma lactate concentration prior to (about 7·8 mmol l^{-1}) and at the end of the EX2 bouts (8·3–8·5 mmol l^{-1}) were higher ($P < 0·05$) than the corresponding values for EX1.

The $V–A_{diff}$ blood lactate prior to EX1 was not different from zero, but during EX1 it increased to 2·6–2·7 mmol l^{-1}. During recovery it declined to 1·9 (P-leg) and 1·6 (A-leg) mmol l^{-1} after 3 min and further to 0·5 (P-leg) and 0·1 (A-leg) mmol l^{-1} after 10 min. Prior to EX2 the $V–A_{diff}$ lactate was 1·9 (P-leg) and 1·5 (A-leg) mmol l^{-1}, and it was unaltered during EX2. The values at the end of EX2 were lower ($P < 0·05$) than the corresponding values in EX1.

The net lactate release of 11–12 mmol min^{-1} at the end of EX1 declined to 4·8 and 5·5 mmol min^{-1} after 3 min of recovery for the P- and the A-leg, respectively (Fig. 2). After 10 min there was still ($P < 0·05$) a net release for the P-leg, while the net release for the A-leg was not different from zero ($P > 0·05$). Throughout recovery no differences ($P > 0·05$) were observed between the P- and A-leg. The lactate release

was 4·4 (P-leg) and 4·8 (A-leg) mmol min^{-1} before EX2, and during EX2 it increased to 8·2 and 9·0 mmol min^{-1}, respectively, which was lower ($P < 0·05$) compared to EX1.

The muscle lactate increased from 1·0–1·3 mmol (kg wet wt)$^{-1}$ to about 23 mmol (kg wet wt)$^{-1}$ during EX1. Thereafter it decreased to 11·2 (P-leg) and 13·4

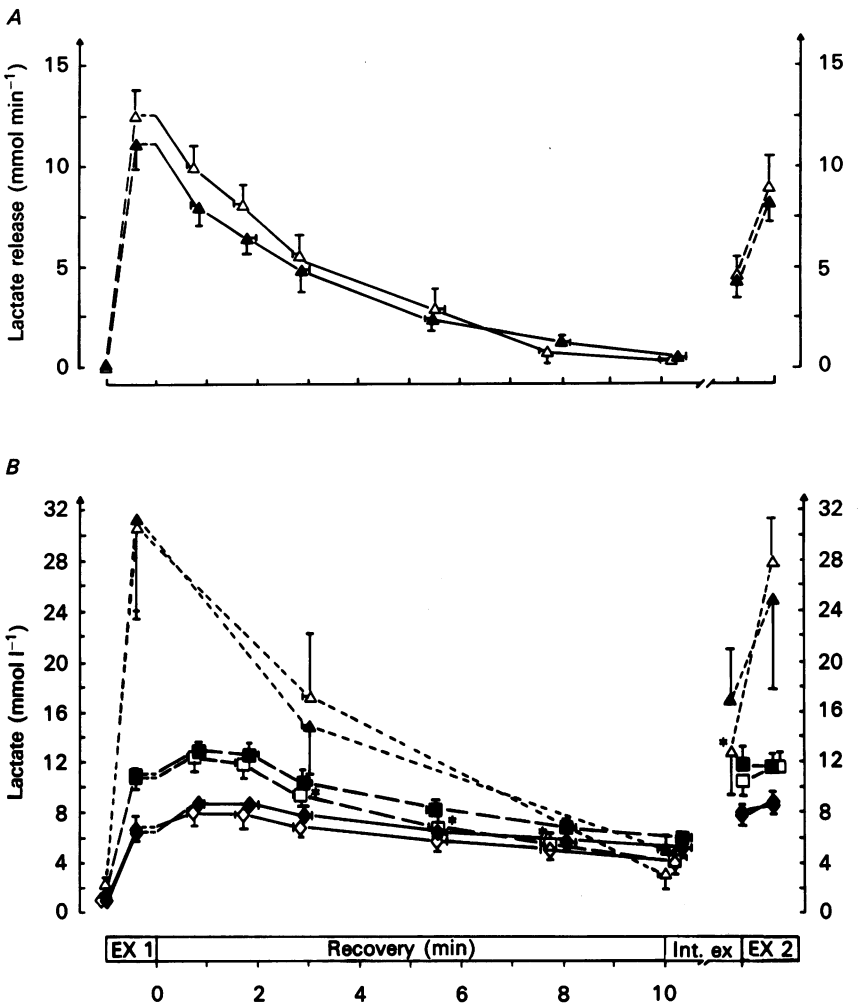

Fig. 2. Lactate responses during exercise and recovery. (Int. ex., intermittent exercise). *A*, lactate release for the P-leg (filled symbols) and the A-leg (open symbols). *B*, femoral arterial (diamonds) and venous (squares) plasma lactate concentrations and intracellular muscle lactate concentrations (triangles). * Significant difference ($P < 0·05$) between P-leg and A-leg.

(A-leg) mmol (kg wet wt)$^{-1}$ during the first 3 min of recovery, and to 4·4 and 3·2 mmol (kg wet wt)$^{-1}$, respectively, during the next 7 min of recovery. The latter two values were different from each other ($P < 0·05$). The muscle lactate

concentration for the P-leg of 13·1 mmol (kg wet wt)$^{-1}$ prior to EX2 was also higher ($P < 0·05$) compared to the A-leg (9·9 mmol (kg wet wt)$^{-1}$), but the lactate concentrations after EX2 of 18·5 (P-leg) and 20·4 (A-leg) mmol (kg wet wt)$^{-1}$ were not different from each other and were similar to those in EX1.

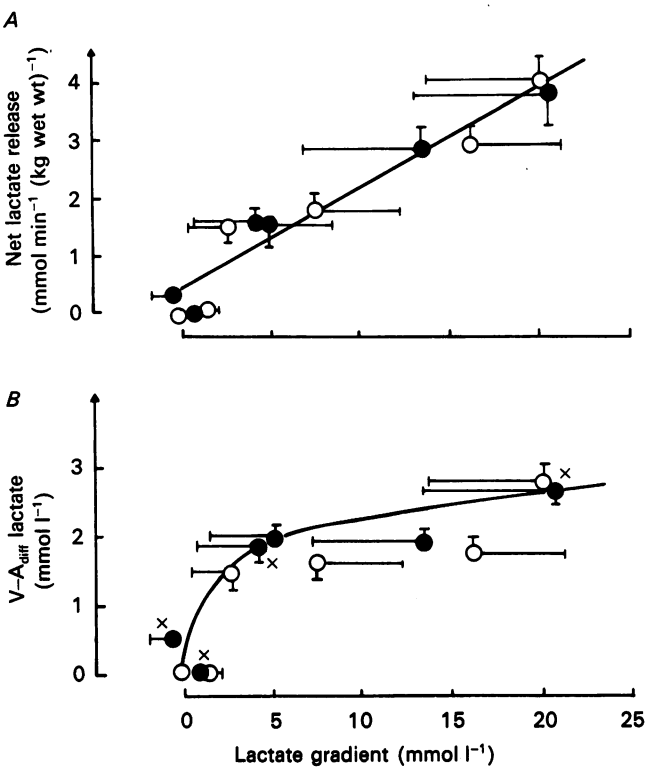

Fig. 3. The relationship between the muscle lactate gradient and lactate exchange. In *B*, the venous–arterial difference (mmol l^{-1}) for lactate is presented while the net lactate release (mmol min^{-1} kg^{-1}) is summarized in *A*. The line for net lactate release is obtained by linear regression, and the curve for net V–A$_{diff}$ lactate is drawn by eye based on the values obtained for the passive leg prior to the intense, intermittent exercise (marked with ' × '). Open symbols, A-leg; filled symbols, P-leg.

The muscle water content increased from 76–77 to 78–79% during EX1, and it was about 78% throughout the following 10 min of recovery. Prior to EX2 it was 78–79% and no change occurred during EX2. The intracellular concentration of lactate was about 31 mmol l^{-1} at the end of the EX1 bouts, and it decreased to 5·0 (P-leg) and 3·9 (A-leg) mmol l^{-1} after 10 min of recovery (Fig. 2). The intracellular lactate concentration of the P-leg prior to EX2 of 16·7 mmol l^{-1} was higher ($P <$ 0·05) than for the A-leg (12·8 mmol l^{-1}), but no differences were observed at the end of EX2. When all the data are examined the muscle lactate gradient (intracellular lactate concentration – femoral venous plasma lactate concentration) was linearly related ($r = 0·96$; $P < 0·05$) to lactate efflux, while its relationship to V–A$_{diff}$ lactate

began to level off at high gradients (Fig. 3). Thus, high blood flow was the cause of the maintenance of the linear relationship with efflux despite the moderation in the $V-A_{diff}$.

Inverse individual relationships between the percentage of ST fibres and muscle lactate at the end of EX1 were obtained for either leg ($r = -0.79$ (P-leg) and $r = -0.90$ (A-leg); $P < 0.05$). Correspondingly the percentage of ST fibres was inversely correlated to the muscle lactate gradient at every sampling time. However, the percentage of ST fibres was not related to the lactate release at the end of EX1 when either expressed in mmol min^{-1} or mmol min^{-1} (kg muscle)$^{-1}$. The number of capillaries per ST, FTa and FTb fibre were 4·63 (range, 4·34–4·93), 4·21 (3·95–4·47) and 4·07 (3·51–4·92), respectively, and the mean number of capillaries per fibre was 1·98 (1·75–2·19). None of these was related to the muscle lactate gradient or lactate efflux either during exercise or recovery.

Muscle and blood acid–base balance

Muscle H^+ concentration

The muscle H^+ concentration at the end of the EX1 bouts of about 188 nmol l^{-1} (corresponding pH, 6·73) decreased during recovery to 118 (6·92) and 151 (6·82) nmol l^{-1} after 3 min and further to 87 (7·06) and 102 (6·99) nmol l^{-1} after 10 min for P-leg and A-leg, respectively. The latter values were not different from the values prior to EX1 (about 90 nmol l^{-1}; pH, 7·04). The muscle H^+ concentrations before EX2 were elevated to 143 (6·84) and 141 (6·85) nmol l^{-1}, respectively, and they increased to 168 (P-leg) and 170 (A-leg) nmol l^{-1} during EX2.

The H^+ gradient (intracellular H^+ concentration – femoral venous H^+ concentration) was 134–135 nmol l^{-1} at the end of EX1, and it decreased during recovery to 77 (P-leg) and 102 (A-leg) nmol l^{-1} after 3 min. After 10 min of recovery the values (48–60 nmol l^{-1}) were similar to those before EX1. Prior to EX2 the H^+ gradient was elevated to 93–94 nmol l^{-1} and it increased further to 117–123 nmol l^{-1} at the end of EX2.

Blood H^+, ABE, P_{CO_2} and HCO_3^- (Figs 4 and 5)

The venous blood H^+ concentration of about 70 nmol l^{-1} (pH, 7·14–7·15) at the end of the EX1 bouts was maintained during early recovery, and then, it decreased to about 60 nmol l^{-1} after 3 min (Fig. 4). A further decline was observed during the rest of recovery with no difference between the P- and A-leg, and the values after 10 min were similar to those before EX1 (42–43 nmol l^{-1}). The venous H^+ concentrations prior to the two EX2 bouts were elevated to 60·4 (P-leg) and 56·9 (A-leg) nmol l^{-1}, and did not change during EX2. Thus, the values at the end of the EX2 bouts were lower ($P < 0.05$) compared to EX1. The arterial blood H^+ concentration at the end of EX1 was similar to rest, but during the first 3 min of recovery it increased to approximately 60 nmol l^{-1}. Then, it decreased to resting levels after 10 min. Prior to EX2 the arterial blood H^+ concentrations of 46–47 nmol l^{-1} were higher ($P < 0.05$) than before EX1, but during EX2 they returned to resting level.

The arterial and venous ABE were about −1·0 and −0·2 mmol l^{-1}, respectively, prior to EX1, and both decreased during exercise being about −5 and −6 mmol l^{-1}, respectively, at the end of EX1 (Fig. 4). A further decline was observed during early

recovery, and the venous ABE decreased more than the arterial values. Thus, the $V-A_{diff}$ ABE decreased to $-2{\cdot}8$ (P-leg) and $-2{\cdot}2$ (A-leg) mmol l^{-1} after 0·8 min of recovery. The arterial ABE for both the P- and A-leg was rather constant during the remaining part of the 10 min of recovery, while the venous ABE values increased.

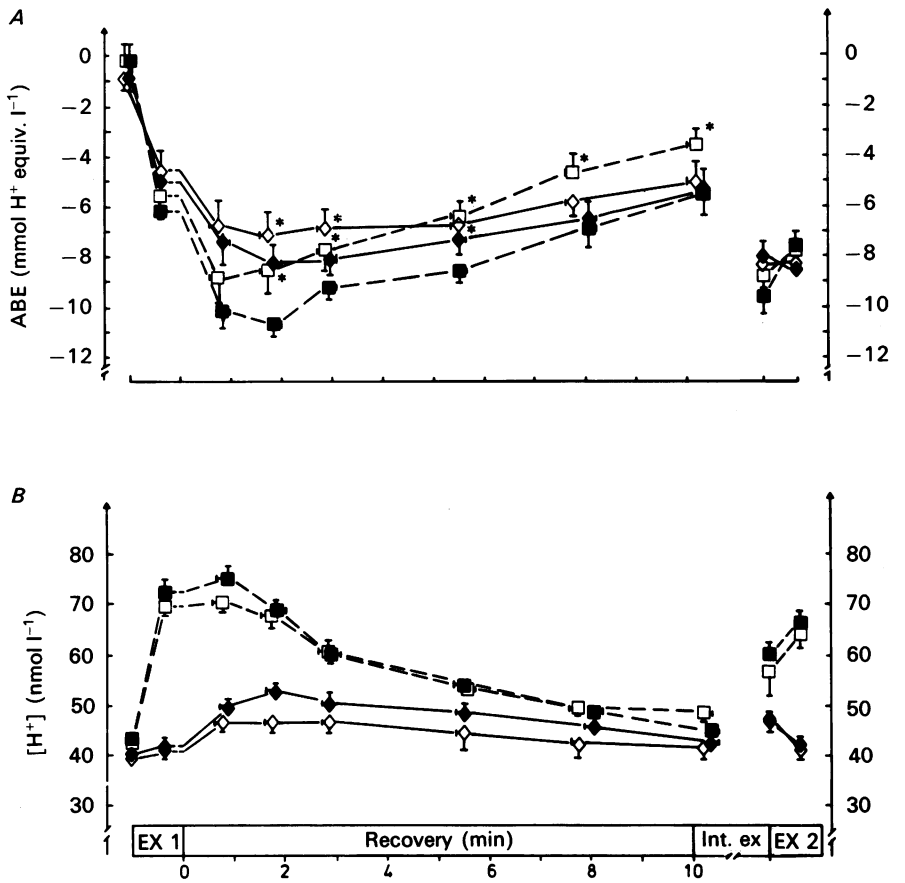

Fig. 4. The responses of H^+ and actual base excess (ABE) to exercise and recovery. Femoral arterial (diamonds) and venous (squares) ABE (*A*) and blood H^+ concentration (*B*) for the P-leg (filled symbols) and the A-leg (open symbols). *Significant difference ($P < 0{\cdot}05$) between P-leg and A-leg.

After 5·5 min the venous ABE for the A-leg became higher than the arterial ABE, and the $V-A_{diff}$ was 1·5 mmol l^{-1} after 10 min, as a result of the buffering related to the reduction of Hb in venous blood. The arterial and venous ABE for the P-leg were $-5{\cdot}4$ and $-5{\cdot}5$ mmol l^{-1}, respectively, after 10 min, and both were lower ($P < 0{\cdot}05$) than at rest. The intense intermittent exercise resulted in a considerable decrease in both arterial and venous ABE, with no change in arterial, but a slight increase in venous ABE during EX2.

The femoral venous blood P_{CO_2} of 77–79 mmHg at the end of the EX1 bouts decreased during recovery, particularly for the P-leg (Fig. 5). Thus, P_{CO_2} for this leg

was lower ($P < 0.05$) than for the A-leg after 1·8 min and until 10 min of recovery, when values (41–42 mmHg) similar to rest were obtained for both legs. Venous P_{CO_2} prior to EX2 was 47·1 (P-leg) and 52·7 (A-leg) mmHg, and it increased during EX2 to 64·2 and 57·1 mmHg, respectively, which was lower ($P < 0.05$) than at the end of

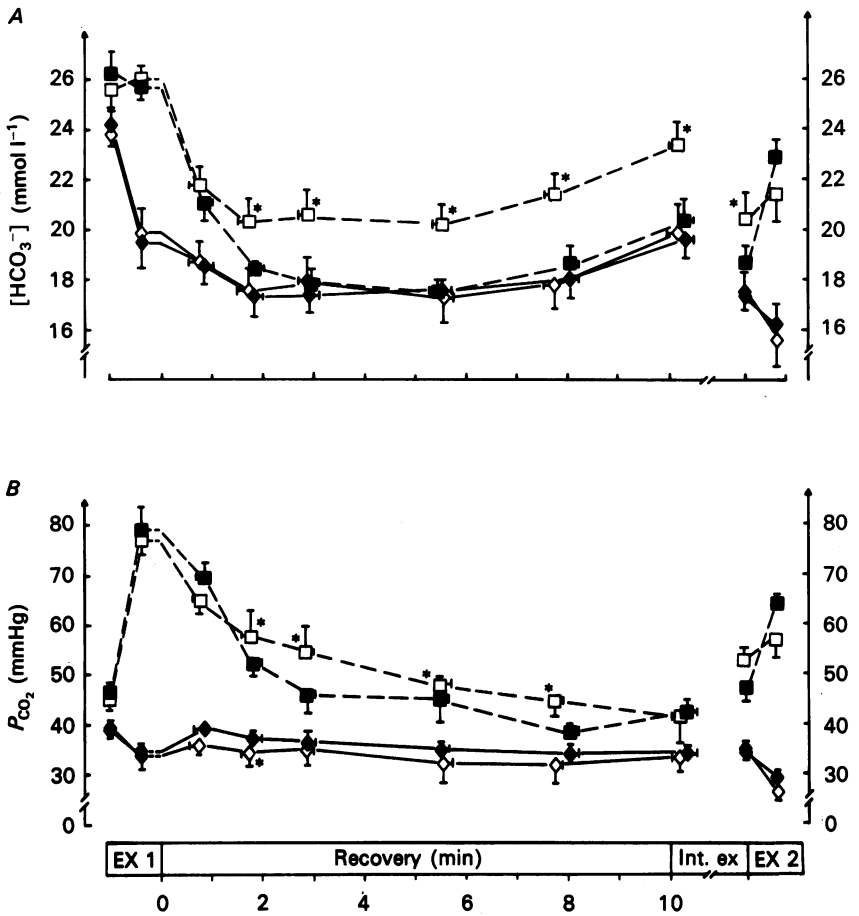

Fig. 5. Summary of the P_{CO_2} and HCO_3^- data for the leg. Femoral arterial (diamonds) and venous (squares) HCO_3^- concentration (*A*) and plasma P_{CO_2} (*B*) for the P-leg (filled symbols) and for A-leg (open symbols). *Significant difference ($P < 0.05$) between P-leg and A-leg.

the EX1. The arterial blood P_{CO_2} at the end of the EX1 changed only slightly during the 10 min recovery, and remained lower ($P < 0.05$) than prior to EX1 (Fig. 5). Before the EX2 bouts it was about 34 mmHg, and the values at the end of EX2 were lower compared to EX1.

At the end of the EX1 bouts the femoral venous blood HCO_3^- concentrations were 26 mmol l^{-1} and they were similar to rest (Fig. 5). During recovery they decreased, particularly for the P-leg. Thus, the venous blood HCO_3^- concentration after 1·8 min was lower ($P < 0.05$) than for the A-leg, and the difference was maintained during the

remaining part of recovery. After 10 min the venous values were still lower ($P < 0.05$) than at rest prior to EX1. The venous HCO_3^- concentration prior to EX2 of 18·6 mmol l^{-1} for the P-leg was lower ($P < 0.05$) than the 20·4 mmol l^{-1} for the A-leg, but no difference was observed at the end of EX2. The arterial blood HCO_3^- concentrations at the end of the EX1 bouts were 19–20 mmol l^{-1}, and they changed only slightly during the 10 min of recovery, but remained lower ($P < 0.05$) than prior to EX1 (about 24 mmol l^{-1}). Before EX2 arterial HCO_3^- was 17·4 mmol l^- for either leg, and it decreased slightly to 15–16 mmol l^{-1} during EX2.

DISCUSSION

This study examined lactate transport and release in the human quadriceps under a variety of circumstances. In agreement with our earlier findings (Juel *et al.* 1990) lactate efflux increased with no sign of levelling off at high muscle lactate concentrations. This is in contrast to Jorfeldt *et al.* (1978), who suggested a maximal efflux at a muscle lactate concentration of 4 mmol (kg wet wt)$^{-1}$. However, their conclusion is misleading as they related lactate release to the muscle lactate concentrations rather than the muscle lactate gradient i.e. the blood lactate concentrations were not considered. When lactate efflux data from this and other studies including that of Jorfeldt *et al.* (1978) are examined relative to the muscle lactate gradient no saturation is apparent (Fig. 6).

Lactate efflux is a product of both flow and transport; the latter is approximated by the $V–A_{diff}$ lactate. In contrast to the relationship for the lactate release, when the $V–A_{diff}$ lactate in the present study is compared to the muscle lactate gradient a partial saturation is apparent (Fig. 3). This is in accordance with findings in *in vitro* studies demonstrating that diffusion across the sarcolemma is dominated by a lactate–H^+ co-transport mechanism, and that simple diffusion also occurs (Juel, 1988; Watt *et al.* 1988; Juel, 1991; Juel & Richter, 1991). Saturation kinetics for lactate have also been observed in mouse diaphragm (Koch, Webster & Lowell, 1981) and dog gracilis muscle (Connett, Gayeski & Honig, 1986). In the present study, the lactate efflux measured is the net release of lactate over all fibre membranes in the muscle. Thus, it is likely that the partial saturation in the $V–A_{diff}$ lactate is a response of no transport saturation in some fibres and partial or total saturation in other fibres with lactate concentrations considerably higher than the Michaelis constant (K_m) for the lactate–proton carrier. The latter has been determined to be 20–30 mmol l^{-1} in both rodent and human skeletal muscles (Watt *et al.* 1988; Juel, 1991; Juel & Richter, 1991). In accordance with other studies (Tesch, 1980) an inverse relationship between the percentage of ST fibres and muscle lactate accumulation was observed, suggesting that the lactate concentration was highest in the FT fibres at the end of exercise in the present study. Thus, it is likely that it was primarily the FT fibres which were saturated. This is supported by the lack of relationship between the percentage of ST fibres and lactate release at the end of EX1 despite the lower muscle lactate gradients in muscle with a greater percentage of ST fibres. It is also in agreement with the observation in *in vitro* studies of rat skeletal muscles that the ST fibres had a higher lactate transport capacity than FT fibres (Juel, Honig & Pilegaard, 1991).

The same $V-A_{diff}$ lactate was associated with muscle lactate gradients of 13–16 mmol l^{-1} at the end of EX2 and with gradients of 3–8 mmol l^{-1} obtained both 3 min after EX1 and after the intermittent exercise (Table 1). This demonstrates that factors other than the lactate gradient are important for the lactate transport.

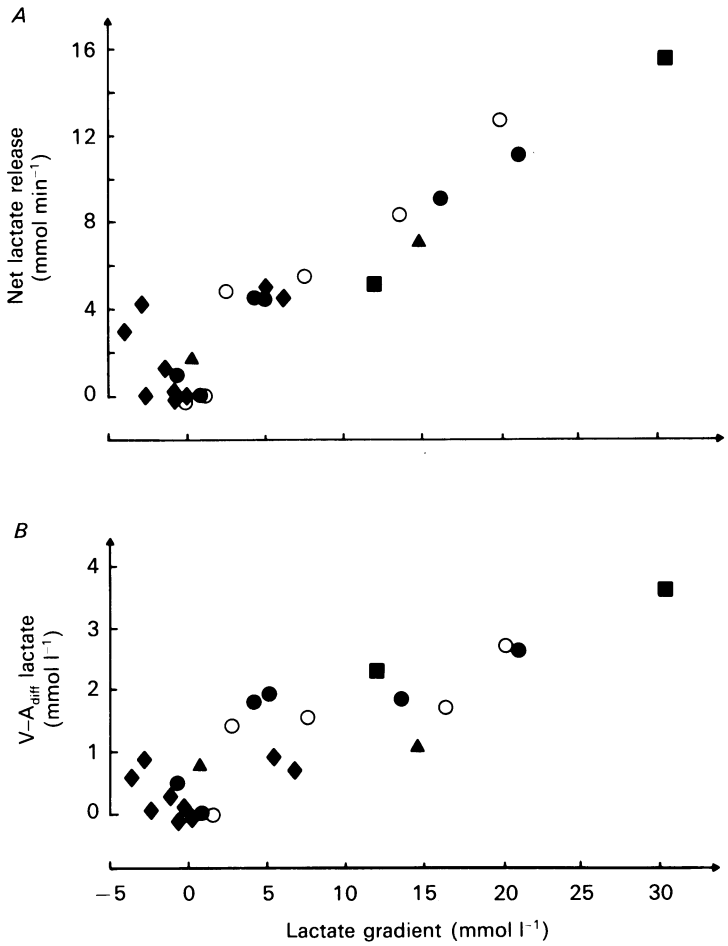

Fig. 6. A comparison of lactate data from the present study with that of others. The lactate release (mmol min^{-1}; *A*) and venous–arterial difference (mmol l^{-1}) for lactate (*B*) are presented in relation to the muscle lactate gradient (mmol l^{-1}) across the sarcolemma for the present study (○, ●) and other studies. The latter are from Jorfeldt *et al.* (1978; ◆), Katz *et al.* (1986; ▲) and Juel *et al.* (1990; ■). In the calculation of the muscle lactate gradient for the study by Jorfeldt *et al.* (1978) and Katz *et al.* (1986) a ratio between whole blood lactate and plasma lactate concentration of 0·5 is used (Juel *et al.* 1990).

By comparing data obtained at these time points the effect of several variables can be examined. Such comparisons have to be taken with caution, since other factors, such as the relative contribution of ionized and unionized lactate to the total transport, might have influenced the lactate release.

TABLE 1. Lactate release and $V-A_{diff}$ for lactate in relation to muscle lactate and H^+ gradient, blood flow and arterial plasma lactate at various measuring points

	Pre-EX1		EX1		Recovery 3 min		Recovery 10 min		Pre-EX2		EX2	
	A	P	A	P	A	P	A	P	A	P	A	P
Lactate release (mmol min^{-1} kg^{-1})	0·00	0·00	4·03	3·84	1·78‡	1·57‡	−0·03	0·34	1·57‖	1·56‖	2·89†	2·85†
$V-A_{diff}$ lactate (mmol l^{-1})	0·02	0·02	2·72	2·63	1·59	1·85	0·05	0·50	1·47	1·93	1·73†	1·90†
Lactate gradient (mmol l^{-1})	1·3	0·9	20·2	20·6	7·6‡§	4·2‡*	−0·2	−0·6	2·5‖	4·9‖	16·3†	13·5†
H^+ gradient (nmol l^{-1})	51	52	135	134	102‡	77‡	60	48	94‖	93‖	123†	117†
Blood flow (l min^{-1} kg^{-1})	0·14	0·14	1·54	1·32	1·12‡	0·82‡*	0·95	0·33*	1·04‖	0·77‖*	1·45	1·53
Arterial plasma lactate (mmol l^{-1})	0·95	0·95	6·32	6·25	6·83‡§	7·60‡	4·17	4·98	7·78	7·87	8·52†	8·28†

* Significant difference between the P- and A-leg.
† Significant difference between EX1 and EX2.
‡ Significant difference between recovery for 3 min and EX2.
§ Significant difference between recovery for 3 min and pre-EX2.
‖ Significant difference between pre-EX2 and EX2.

The finding that the higher muscle lactate and H^+ gradient at the end of EX2 did not elevate the $V-A_{diff}$ lactate in comparison to 3 min of recovery from EX1, suggests that the higher arterial plasma lactate and/or blood flow, or perhaps the muscle contraction per se at the end of EX2 limited the lactate transport (Table 1). Thus, it appears that high arterial lactate concentrations, in addition to reducing the muscle lactate gradient, also had a direct inhibitory effect on lactate efflux from the muscle. Paradoxically, high blood flow was associated with a reduction in $V-A_{diff}$ lactate, and thus, probably the lactate transport across the muscle sarcolemma. This is apparent when the $V-A_{diff}$ lactate for the P- and A-leg is similar at 3 min of recovery from EX1. The lower arterial lactate concentration and higher muscle lactate and H^+ gradients should favour a larger release for the A-leg, but it appears to have been counteracted by the higher blood flow. This is reinforced by the finding of a similar lactate release during the entire passive and active recovery period, although the blood flow was considerably higher during the active recovery.

It is unclear why elevated blood flow would cause lower lactate transport. Diffusive flux depends on the capillary surface area available for exchange and probably also on the rate of perfusion of each capillary. It could be expected that the higher blood flow would improve the wash-out of lactate and protons from the interstitial space, thereby increasing the lactate gradient between the intra- and extracellular compartments. This should promote lactate transport. However, the mean transit time of each capillary at high blood flows might be too long to ensure optimal exchange of lactate between the interstitial and capillary blood. Honig, Odoroff & Frierson (1982) demonstrated that the number of perfused capillaries increased almost to a maximum, when the dog gracilis muscle was stimulated at low frequency. However, when the stimulation frequency was increased further, the number of perfused capillaries was reduced. Thus, the exchange area was reduced and the transit time prolonged.

An alternative explanation would be that blood circulation was arrested in a period during each contraction cycle due to mechanical hindrance (Walløe & Wesche, 1988), and that this diminished the time for optimal exchange of lactate between the interstitium and capillaries. This is supported by our earlier observations that the contraction occupies a substantial fraction (50–60%) of the 1 s duty cycle during intense exercise at work rates similar to those used in the present study, and that the blood flow at such exercise was not elevated in comparison to exercise at a high submaximal intensity (Bangsbo, Gollnick, Graham, Juel, Kiens, Mizuno & Saltin, 1990).

The role of extracellular pH in lactate release has received much attention. *In vivo* studies on isolated muscle preparation have shown that the lactate release is increased during alkalosis and decreases in both metabolic and respiratory acidosis (Mainwood & Worsley-Brown, 1975; Spriet, Matsos, Peters, Heigenhauser & Jones, 1985; Spriet, Lindinger, Heigenhauser & Jones, 1986). In addition, in studies of man it has been observed that blood lactate was inversely related to the degree of extracellular acidosis (Hood, Schubert, Keller & Müller, 1988). In the present study the $V-A_{diff}$ lactate was the same 3 min after EX1, as it was prior to and at the end of EX2, despite considerable differences in the H^+ gradients (Table 1). Thus, any effect on the lactate transport caused by the difference in H^+ gradient must have

been counteracted. It might suggest that transport is minimally affected by the transmembrane proton gradient under conditions when the extracellular pH is not manipulated.

The studies using whole body exercise are characterized by larger arterial lactate concentrations, due to the greater mass of active muscle, and by a smaller $V-A_{diff}$ lactate than in the present study. The high arterial lactate concentration of 12 mmol l^{-1} in the study by Katz *et al.* (1986) might explain the modest $V-A_{diff}$ lactate (1·1 mmol l^{-1}) even though the muscle lactate concentration was high (25 mmol (kg wet wt)$^{-1}$) (Fig. 6). Correspondingly, the finding of a similar lactate $V-A_{diff}$ (0·9 mmol l^{-1}) at muscle lactate concentrations of 3·7 and 9·3 mmol (kg wet wt)$^{-1}$ in the study by Jorfeldt *et al.* (1978) might partly have been caused by the differences in arterial lactate concentration (4·9 and 6·7 mmol l^{-1}, respectively) (Fig. 6). Furthermore, the lower lactate release in those studies at a given muscle lactate gradient in comparison to the present study may also be related to a major limitation in their experimental models. During submaximal bicycle exercise, as used by Jorfeldt *et al.* (1970) and Katz *et al.* (1986), a considerable part of the leg blood flow is perfusing inactive or only partly active muscles, which will take up lactate (Ahlborg, Hagenfeldt & Wahren, 1975). Thus, the true lactate release (as well as the $V-A_{diff}$ lactate) from the biopsied quadriceps muscle will be underestimated. By using the knee-extensor exercise and occluding the perfusion to the lower leg, as in the present study, this problem is minor during intense exercise and early recovery. The underestimation due to uptake by the hamstring and adductor muscles at the end of the intense exercises and 3 min into recovery can be calculated, based on the present data and earlier findings, to be about 0·5 and 0·3–0·4 mmol min^{-1}, respectively, or 4–5 and 7–8 % of the total release (Bangsbo *et al.* 1991). However, as recovery continues and the net release of lactate from the quadriceps muscles decreases, the relative underestimation becomes significant; amounting to about 0·1 mmol min^{-1} after 10 min of recovery.

Lactate efflux is associated with release of H^+. Based on comparison between $V-A_{diff}$ ABE (adjusted for changes in buffering by the reduced Hb) and $V-A_{diff}$ lactate, it appears that the H^+ release exceeds lactate efflux both during exercise and recovery (Fig. 7). The ratios between the change in ABE and lactate were 1·4–1·6 at the end of EX1 bouts and the mean ratios were 1·3 an 1·5 during the subsequent 10 min of passive and active recovery, respectively. Similar findings were obtained in other human studies, but in those experiments the conclusions were based on changes in base excess and lactate accumulation in arterial or arterialized blood (Medbø & Sejersted, 1985). Therefore, it could not be excluded that the findings were a result of unequal uptake rates of H^+ and lactate by non-exercising tissue. This problem is minor in the present study as direct Fick calculations were used and the perfusion of inactive muscles was relatively small as discussed above. Katz *et al.* (1985) performed single venous–arterial measurements after intense intermittent bicycle exercise, and found that the H^+ release was higher than the lactate release during early recovery, but the difference was not significant (Katz *et al.* 1985; Fig. 4). Thus, it appears that the proton release is higher than the lactate efflux for human muscles, but differences between species might exist. A H^+–lactate ratio above one has also been reported for rat muscles (Heigenhauser *et al.* 1985) and for dog muscles

(Chirtel *et al.* 1984). However, the conclusion in the latter studies has been questioned, as it was demonstrated that the buffering due to the reduction of Hb was overestimated in dogs, and a 1:1 relation was proposed (Stainsby & Eitzman, 1988). In addition, a ratio below one for frog muscles has been demonstrated (Mainwood & Worsley-Brown, 1975).

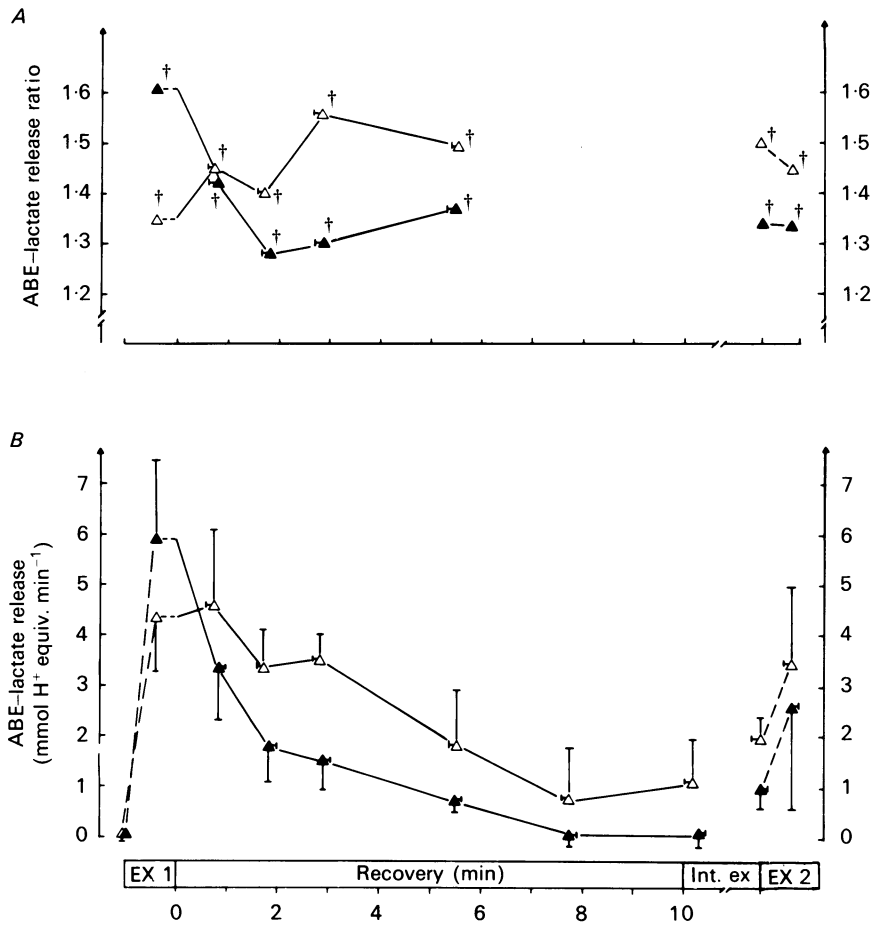

Fig. 7. A comparison of H^+ and lactate release. The figure presents the ratio between ABE and lactate release (*A*) and the difference between H^+ efflux (based on the venous–arterial changes in ABE) and net lactate release (*B*) for the P-leg (filled symbols) and the A-leg (open symbols). The ABE values are adjusted for changes related to the buffering caused by the reduction of Hb. The ratio between ABE and lactate release for values obtained at rest prior to EX1 and during the late part of recovery are not given, as they are uncertain due to the small net release of lactate. †Significantly higher ($P < 0{\cdot}05$) than 1.

The finding by Heisler (1988) of an increasing H^+–lactate ratio with enhancing blood flow to the rat hindlimb, could only partly be supported by the data from the present study. While the blood flow during active recovery both after EX1 and prior to EX2 was higher, the ratios were not significantly elevated compared to passive

recovery (Fig. 7). Despite the slightly higher release of H^+ during the active recovery, no difference in muscle pH was observed. For both the passive and the active leg muscle pH returned to resting level after 10 min of recovery, while the muscle lactate was still significantly elevated. The faster recovery of H^+ is in accordance with other studies (Juel, 1988; Juel *et al.* 1990).

From the data in the present study it appears that hydrogen ions can be extruded from muscle cells independently of any lactate transporter, but the mechanism responsible for this is unclear and cannot be verified from the present data. The proton movement in and out of the muscle cells must have been accompanied by opposite fluxes of cations or co-fluxes of anions to maintain electroneutrality. A likely candidate is the Na^+–H^+ exchange mechanism, which has been shown to be important for pH regulation in mouse muscles both at rest and in recovery from muscle contraction (Juel, 1988).

In summary, lactate transport appears to be partly saturated at high muscle lactate concentrations. However, lactate efflux continues to increase due to enhanced blood flow during intense exercise. Several factors such as arterial lactate concentration, blood flow, muscle contraction and muscle morphology appear to affect muscle lactate transport. Proton release is faster than the lactate efflux both during exercise and recovery.

The study was supported by grants from Team Danmark and Danish Natural Science Foundation (11–7776). Terry Graham was supported by NSERC (Canada) International Collaborative Research Grant.

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