Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man

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1. The aim of this study was to examine the effect of muscle pH on muscle metabolism and development of fatigue during intense exercise.

2. Seven subjects performed intense exhaustive leg exercise on two occasions: with and without preceding intense intermittent arm exercise leading to high or moderate (control) blood lactate concentrations (HL and C, respectively). Prior to and immediately after each exercise bout, a muscle biopsy was taken from m. vastus lateralis of the active leg. Leg blood flow was measured and femoral arterial and venous blood samples were collected before and frequently during the exhaustive exercises.

3. The duration of the exercise was shorter in HL than in C (3·46 ± 0·28 *vs.* 4·67 ± 0·55 min; means ± S.E.M.; $P < 0·05$). Before exercise muscle pH was the same in C and HL (7·17 *vs.* 7·10), but at the end of exercise muscle pH was lower in HL than in C (6·82 *vs.* 6·65; $P < 0·05$). The release of potassium during exercise was higher ($P < 0·05$) in HL compared with C, but the arterial and femoral venous plasma potassium concentrations were the same at exhaustion in HL and C.

4. Muscle lactate concentration was higher in HL compared with C (3·7 ± 0·4 *vs.* 1·6 ± 0·2 mmol (kg wet weight)$^{-1}$; $P < 0·05$), but the same at exhaustion (26·5 ± 2·7 *vs.* 25·4 ± 2·4 mmol (kg wet weight)$^{-1}$). Total release of lactate in HL was lower than in C (18·7 ± 4·5 *vs.* 50·4 ± 11·0 mmol; $P < 0·05$), but rate of lactate production was not different (9·0 ± 1·0 *vs.* 10·2 ± 1·3 mmol (kg wet weight)$^{-1}$ min^{-1}). The rate of muscle glycogen breakdown was the same in C and HL (8·1 ± 1·2 *vs.* 8·2 ± 1·0 mmol (kg wet weight)$^{-1}$ min^{-1}).

5. The present data suggest that elevated muscle acidity does not reduce muscle glycogenolysis/glycolysis and is not the only cause of fatigue during intense exercise in man. Instead, accumulation of potassium in muscle interstitium may be an important factor in the development of fatigue.

Intense exercise is associated with a high production of lactate with concomitant elevated acidity within the exercising muscles. This may affect the function of the muscle cells, as it is known from *in vitro* studies that low pH has an inhibitory effect on several reactions within the muscle cell (Danforth, 1965; Fabiato & Fabiato, 1978; Chasiotis, Hultman & Sahlin, 1983; Lännergren & Westerblad, 1991). Thus, lactic acid accumulation and lowered pH may cause fatigue during intense exercise (see Sahlin, 1986). However, recent *in vivo* studies using repeated intense exercise suggest that lactate and pH may not be exclusive determinants of fatigue (Bangsbo, Graham, Johansen, Strange, Christensen & Saltin, 1992*a*; Bangsbo, Graham, Kiens & Saltin, 1992*b*) defined as a failure to maintain the required power output (Edwards, 1981). Instead, it has been speculated that a progressive accumulation of potassium in the interstitium during intense exercise may be implicated in the fatigue process (Sjøgaard, 1990; Bangsbo *et al.* 1992*a*, *b*).

It has been demonstrated in several studies that muscle lactate accumulation and production are reduced when intense exercise is repeated (Spriet, Lindinger, McKelvie, Heigenhauser & Jones, 1989; Bangsbo *et al.* 1992*a*, *b*). This could be explained by an impaired glycolysis due to successive increase in the muscle H^+ concentration prior to the subsequent exercise bouts, since it has been demonstrated that lowered pH has an inhibitory effect on the activity of phosphorylase and phosphofructokinase (PFK), which are considered the key regulating enzymes of the glycogenolytic and glycolytic pathway, respectively (Danforth, 1965; Chasiotis *et al.* 1983; Amorena, Wilding, Manchester & Roos, 1990). However, it has also been observed that the rate of lactate production was significantly reduced when intense exercise was repeated

after 1 h of recovery, although muscle lactate was at resting level prior to, and lower at the end of, the second exercise (Bangsbo *et al.* 1992*b*). Therefore, it is questionable whether lowered pH during intense exercise plays a major role in the regulation of glycogenolysis and glycolysis *in vivo*. However, in the studies using repeated high intensity exercise, it is difficult to separate the effect of the elevated acidity from the effect of the previous exercise.

The role of pH in the development of muscle fatigue and in regulation of muscle metabolism during intense exercise may be examined by performing such exercise after intense exercise with other muscle groups. The latter exercise may elevate the lactate concentration in the blood and muscles prior to the exercise (Karlsson, Bonde-Petersen, Henriksson & Knuttgen, 1975; Bangsbo, Aagaard, Olsen, Kiens, Turcotte & Richter, 1995) and affect the exchange of lactate and H^+ during the exercise and thus the muscle lactate and pH level during exercise. Such an exercise regime has been used in several studies and a reduction in performance has been observed when exercise is performed with some muscle groups after exercise with other muscle groups (Hogan & Welch, 1984; Jacobs, Hermiston & Symons, 1993), but it is unclear what causes the reduction in performance. Muscle biopsies have been obtained prior to and after intense exhaustive exercise with one muscle group with and without prior exercise with another muscle group (Karlsson *et al.* 1975). The accumulation of lactate in the muscle appeared to be the same, whether cycle exercise was preceded by arm exercise or not. However, only four subjects were studied and muscle pH as well as exchange of metabolites and ions between muscle and blood were not measured.

Thus, the aim of the present study was to examine the influence of muscle pH on the development of fatigue and the metabolic response during intense exercise. Seven subjects performed intense exhaustive leg exercise on two occasions. On one occasion the exercise was preceded by intense intermittent arm exercise and on another occasion no arm exercise was performed before the leg exercise.

METHODS

Subjects

Seven, healthy, habitually physically active male university students with no history of cardiovascular disease, clotting disorders or other abnormalities ranging in age from 19 to 28 years, with an average height of 178 cm (range, 173–185 cm) and an average body mass of 69·3 kg (range, 63·1–78·2 kg) participated in the experiment. Four of the subjects had participated in previous experiments of similar design to the present study. The maximum oxygen uptake ($\dot{V}_{O_2,max}$) of the subjects was 4·26 l min^{-1} (range, 3·72–4·93 l min^{-1}). The subjects were fully informed of any risks and discomforts associated with these experiments before giving their informed oral consent to participate. Honorarium was provided to the subjects according to an hourly rate for student employees. The study conforms with the code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local Ethics committee. Subjects were covered by state medical insurance and in addition by the same insurance as covers hospitalized patients in case of complications.

Procedures

Subjects performed one-legged exercise in the supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscle (Andersen & Saltin, 1985). They also performed arm cranking in an upright sitting position (see below). The subjects practised the exercise on more than four separate occasions, and in the final pre-experiment the subjects completed the entire protocol for the experiments (see below) except for the invasive measurements.

Protocol

About 3 h before an experiment the subjects had a light breakfast consisting of a few slices of bread with marmalade and 300–500 ml juice. They reported to the laboratory about 2 h prior to the experiment. After changing they rested 30 min in the supine position in an room specially arranged for invasive procedures. Using aseptic techniques a catheter was then placed in the femoral artery of the experimental leg under local anaesthesia. The tip was positioned 1–2 cm proximal to the inguinal ligament. A catheter was also placed in the femoral vein of the experimental leg with the tip of the catheter positioned about 1–2 cm distal to the inguinal ligament. A thermistor for measurement of venous blood temperature was inserted through the catheter and was advanced 8–10 cm proximal to the tip. These invasive procedures have been performed in many previous studies and the catheters were removed after about 5 h, which is shorter than, or of similar duration to, previous studies (Bangsbo *et al.* 1992*a*, *b*). The procedures were performed by a medical doctor with more than 10 years of experience in catheterization and in obtaining muscle biopsies. The doctor was present in the room throughout the entire experiment.

After about 1 h of rest in the supine position, the subjects were moved to the experimental room. Then, they performed 10 min of warm-up with the experimental leg at an intensity of 10 W. After 33 min during which the subjects either rested or performed intense arm exercise (see below) the subjects performed exhaustive knee-extensor exercise with the experimental leg (61·4 ± 3·7 W, mean ± S.E.M.; kick frequency, 60 r.p.m.). The force of each kick and its rate were measured continuously and a drop in kick frequency to 55 r.p.m. was the objective determinant for terminating the exercise. The exercise was performed on two occasions separated by 7–14 days. On one occasion intense arm exercise was performed between the warm-up and the exhaustive leg exercise to elevate the blood lactate concentrations (high lactate, HL) and on the other occasion no arm exercise was performed (control, C). The same leg was exercising in the C and HL experiments, and the experiments were performed in a randomized order. In HL the subjects performed arm cranking in an upright position at an intensity of 137 ± 3 W (frequency, 60 r.p.m.). The arm exercise started 3 min after the warm-up with the leg and consisted of four 1-min exercise periods and one 1·5-min period in 30 s intervals. After 4·5 min of rest and three 1-min arm exercise periods separated by 5 min of rest and after a final 5 min rest period the exhaustive leg exercise was performed.

A muscle biopsy was taken prior to and immediately after the exhaustive exercise bout in each condition (C and HL). Furthermore, blood was drawn simultaneously from the femoral artery and vein at rest, before and frequently during the

exhaustive exercise. In addition, blood flow was measured immediately prior to blood sampling. An occlusion cuff placed just below the knee was inflated (220 mmHg) during the exhaustive exercise. The electrocardiogram and arterial blood pressure were continuously monitored and displayed.

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 into the femoral vein for 10–15 s to achieve changes in blood temperature of 0·8–1·0 °C.

Blood analysis

Oxygen saturation of blood and haemoglobin concentration were determined spectrophotometrically (Radiometer OSM-2 Hemoximeter, Copenhagen, Denmark). The hemoximeter was calibrated spectrophotometrically by the cyanomethaemoglobin method (Drabkin & Austin, 1935). Haemoglobin 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). Haematocrit determinations were made in triplicate, using microcentrifugation. P_{O_2}, P_{CO_2} and pH were measured with the Astrup technique (ABL 30, Radiometer). Lactate and glucose were analysed using a lactate and glucose analyser (model 23, Yellow Springs Instruments, Yellow Springs, OH, USA). A part of each blood sample was centrifuged rapidly, and the plasma was collected and stored at −20 °C until analysed. Plasma potassium concentration was measured using a flame photometer (Radiometer FLM3) with lithium as internal standard.

Muscle mass

The mass of quadriceps femoris muscles was estimated based on Simpson's rule, which included measurements of thigh length, multiple circumferences 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. The present subjects had a mean knee-extensor mass of 3·0 kg, with a range of 2·7–3·4 kg.

Muscle biopsies

Muscle samples were analysed for total water content by weighing the samples before and after freeze drying. They were subsequently analysed for lactate, glycogen and creatine phosphate (CP) by fluorometric assays (Lowry & Passonneau, 1972). ATP and inosine monophosphate (IMP) concentrations were determined with a reverse-phase HPLC technique (Tullson, Whitlock & Terjung, 1990). Muscle pH was measured by a small glass electrode (Radiometer GK2801) after homogenizing the freeze-dried muscle sample in a non-buffering solution containing 145 mM KCl, 10 mM NaCl and 5 mM iodoacetic acid (Parkhouse, McKenzie, Hochachaka & Ovalle, 1985). The remainder of the muscle was analysed enzymatically for glucose 1-phosphate (G-1-P), glucose 6-phosphate (G-6-P), and fructose 6-phosphate (F-6-P) by fluorometric assays (Lowry & Passonneau, 1972).

Calculations

Determination of blood CO_2 content. Arterial and venous whole blood CO_2 content was determined from blood haemoglobin, temperature, saturation, pH, and P_{CO_2} according to the calculation described by Douglas, Jones & Reed (1988).

Leg $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and substrate exchange. $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, net lactate, net glucose, and net potassium exchange by the thigh were calculated by multiplying the blood flow or, for the last variable, plasma flow, by the difference between femoral artery and venous ($a-v_{diff}$) concentrations of the variables. For each individual, leg blood flow was determined as the average of the measurements obtained in C and in HL at each time point. This appears valid as only small variations in leg blood flow were found between C and HL ($< 0{\cdot}5$ l min^{-1}) and no systematic or significant differences were observed. 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 leg respiratory quotient (RQ) was calculated as the ratio between the leg $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$.

Total V_{O_2}, total V_{CO_2} and total exchange of substrates. The total V_{O_2}, V_{CO_2}, net lactate, net glucose, and net potassium exchange during the exhaustive exercise are given by the time integral:

$$\int_0^x f(t)\mathrm{d}t,$$

where x is the time of exhaustion, and $f(t)$ is exchange of one of the variables at a given time (t) during recovery. In practice, the exchanges were determined as the areas under $f(t)$ curves, with time on the X-axis. The curves were produced on the assumption that there was a linear relationship with time in the interval between two consecutive measured values.

A fraction of the blood in the femoral vein drains the hamstring and adductor muscles, which may influence the calculated exchange of substances from the quadriceps muscle. The problem is significant for lactate, since the arterial lactate concentration increases considerably during exercise, and inactive muscles extract lactate (Bangsbo *et al.* 1995). An uptake of lactate by the hamstring/adductor muscles will make the lactate efflux calculated from $a-v_{diff}$ lactate and blood flow an underestimation of the true release of lactate from the quadriceps muscle. In order to take this exchange of lactate into account the net uptake of lactate by the hamstring/adductor muscles was calculated from the arterial lactate concentrations during the exercise in the present study and data obtained in another study in which the uptake of lactate by inactive muscles was determined at various arterial lactate concentrations (Bangsbo *et al.* 1995). Thus, it is assumed that the hamstring/adductor muscles responded in a similar fashion as inactive muscles at the same arterial concentrations of lactate. It was, furthermore, assumed that the mass of the hamstring/adductor muscles accounted for 40% of the mass of the muscles in the thigh (Bischoff, 1863).

Changes in muscle variables were calculated on a dry weight basis, and normalized to the water content of resting muscle, since the muscle mass was determined at rest. This made the exchanges of substrates between femoral blood and the quadriceps muscles (expressed per kilogram of active muscle mass) comparable with the muscle values. The net lactate production by the quadriceps muscle during the exercises was calculated as the sum of lactate accumulation and the total net release of lactate (corrected for the lactate uptake by the hamstring/adductor muscles).

Statistics. Differences between C and HL, and differences between the obtained values and measures at rest or nil were determined by the Wilcoxon rank test for paired data (Pratt's modification). A significance level of 0·05 was chosen. Standard error of the mean (S.E.M.) is only given in the text where this value cannot be obtained from a figure or a table.

RESULTS

Exercise duration and work

The duration of the exercise was shorter ($P < 0{\cdot}05$) in HL than in C (3·46 ± 0·28 *vs.* 4·67 ± 0·55 min). Thus, in HL the total work performed was less ($P < 0{\cdot}05$) than in C (12·6 ± 0·95 *vs.* 17·4 ± 2·5 kJ).

Aerobic energy metabolism

The leg blood flow was 0·45 ± 0·04 l min^{-1} before exercise. It increased to 2·22 ± 0·33, 3·22 ± 0·33 and 3·62 ± 0·29 l min^{-1} after 0·5, 1·5 and 2·5 min of exercise, respectively. Leg blood flow just before exhaustion was 4·16 ± 0·38 and 3·91 ± 0·35 l min^{-1} in C and HL, respectively (n.s.).

During exercise the venous oxygen content decreased rapidly whereas the arterial oxygen was unaltered in both C and HL (Fig. 1). Thus, leg $\dot{V}_{O_2}$ increased rapidly and in a similar fashion in C and HL, reaching the same peak $\dot{V}_{O_2}$ at the end of exercise (583 and 574 ml min^{-1}; Fig. 1). The total leg V_{O_2} was 2078 ± 510 (C) and 1452 ± 196 ml (HL) (n.s.).

The estimated leg $\dot{V}_{CO_2}$ increased during exercise with no difference between C and HL, the values at exhaustion being 644 (C) and 720 ml min^{-1} (HL) (Fig. 1). The total leg V_{CO_2} was 2432 ± 563 (C) and 1863 ± 282 ml (HL) (n.s.). The ratio between total leg V_{CO_2} and leg V_{O_2} was 1·19 ± 0·06 and 1·26 ± 0·11 in C and HL, respectively (n.s.).

Anaerobic energy metabolism

Muscle CP concentration decreased similarly during the exercise in the two trials (Table 1). Muscle ATP concentration decreased by 15–25% in both C and HL (Table 1). The increase in muscle IMP concentration was also the same for C and HL (Table 1).

Muscle lactate concentration prior to exercise was lower ($P < 0{\cdot}05$) in C than in HL, but no difference was observed after exercise (Table 1). The rate of lactate accumulation was the same in C and in HL (6·54 ± 1·08 *vs.* 6·99 ± 0·86 mmol (kg wet wt)$^{-1}$ min^{-1}).

As a result of the arm exercise in HL the arterial blood lactate concentration before the exhaustive exercise was

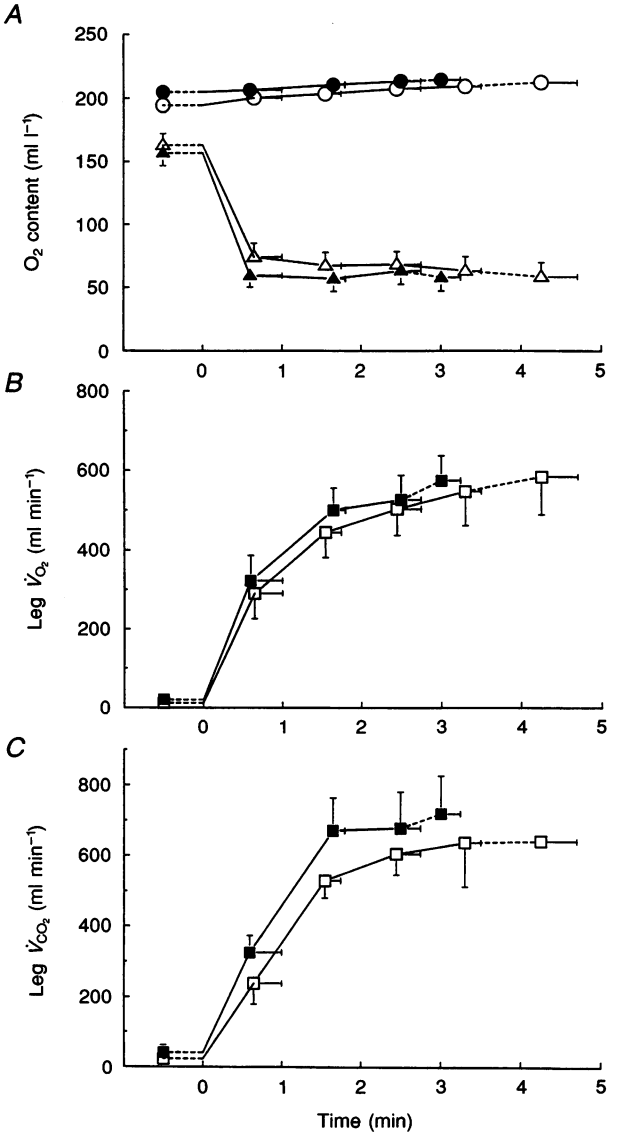

Figure 1. Leg respiratory measurements

Arterial (circles) and femoral venous (triangles) oxygen content (*A*), leg oxygen uptake ($\dot{V}_{O_2}$, *B*) and leg carbon dioxide release ($\dot{V}_{CO_2}$, *C*) during intense exhaustive exercise without (C, open symbols) and with (HL, filled symbols) prior intense intermittent arm exercise. Values after the dashed lines mark measurements just before exhaustion. * Significantly different from C.

Table 1. Muscle metabolite (mmol (kg wet wt)$^{-1}$) and H^+ (nmol l^{-1}) concentrations as well as water content (% H_2O) before (Pre) and after (Post) exhaustive exercise without (C) and with (HL) prior arm exercise

	C		HL	
	Pre	Post	Pre	Post
Glycogen	147·6 ± 17·9	101·6 ± 16·2	135·2 ± 10·7	98·8 ± 12·0
G-1-P	0·11 ± 0·02	0·30 ± 0·03	0·10 ± 0·02	0·29 ± 0·07
G-6-P	0·29 ± 0·04	2·11 ± 0·37	0·37 ± 0·05	3·27 ± 0·72*
F-6-P	0·03 ± 0·01	0·25 ± 0·05	0·05 ± 0·01	0·40 ± 0·16*
Lactate	1·6 ± 0·2	26·5 ± 2·7	3·7 ± 0·4*	25·4 ± 2·4
CP	21·7 ± 6·5	6·5 ± 1·9	21·3 ± 1·1	6·0 ± 1·0
ATP	5·81 ± 0·18	4·31 ± 0·28	6·04 ± 0·13	4·26 ± 0·10
IMP	0·04 ± 0·02	0·81 ± 0·25	0·03 ± 0·02	1·08 ± 0·09
H^+ (nmol l^{-1})	67·9 ± 1·5	153·2 ± 18·6	79·2 ± 6·6	221·7 ± 27·8*
H_2O (%)	75·5 ± 0·4	78·1 ± 0·5	75·1 ± 0·4	77·8 ± 0·5

* Significantly different from the corresponding value in C.

about 11 times higher ($P < 0·05$) in HL than in C, and it remained higher ($P < 0·05$) throughout the exercise (Fig. 2). In HL a lactate uptake was observed before exercise which after the initial phase of exercise turned to lactate release (Fig. 2). In C there was an efflux of lactate during exercise, which was higher ($P < 0·05$) than in HL. The total release of lactate during exercise in C was higher ($P < 0·05$) than in HL (50·4 ± 11·0 *vs.* 18·7 ± 4·5 mmol) and this was also the case when 'normalized' for the shorter exercise time in HL (33·4 ± 6·7 and 17·7 ± 4·3 mmol, respectively; $P < 0·05$).

The uptake of lactate by the hamstrings/adductor muscles during the exercise period was estimated to be 1·5 ± 0·0 (C) and 2·6 ± 0·1 mmol (HL) (see Methods). The total net lactate production (corrected for the uptake by the hamstrings/adductor muscles) in C was 45·6 mmol (kg wet wt)$^{-1}$, which was higher ($P < 0·05$) than in HL (30·5 mmol (kg wet wt)$^{-1}$), but the mean rate of lactate production in C and HL was not different (10·2 ± 1·3 *vs.* 9·0 ± 1·0 mmol (kg wet wt)$^{-1}$ min^{-1}, $P > 0·05$).

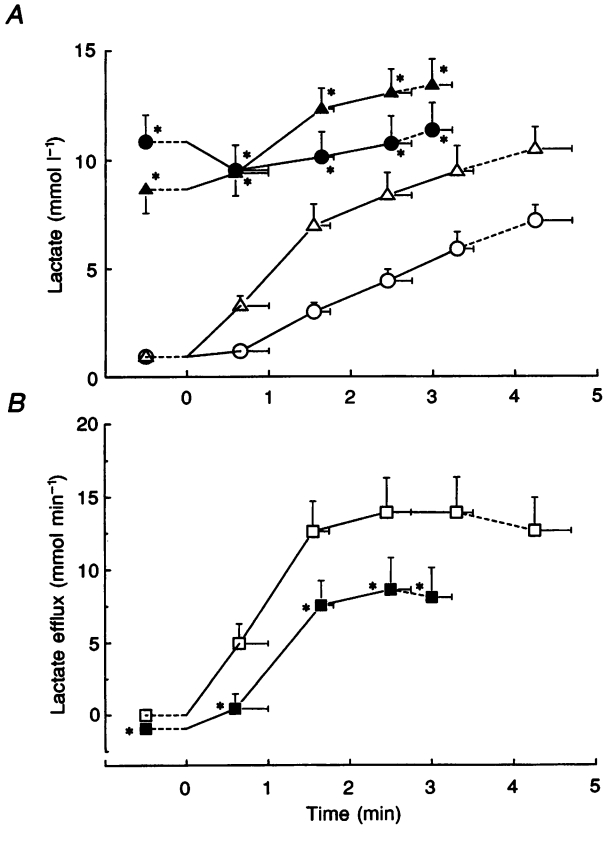

Figure 2. Blood lactate and lactate efflux

Arterial (circles) and venous (triangles) blood lactate concentration (*A*) and lactate efflux (*B*) during intense exhaustive exercise without (C, open symbols) and with (HL, filled symbols) prior intense intermittent arm exercise. Values after the dashed lines mark measurements just before exhaustion. * Significantly different from C.

Total energy production

The total energy production during the exercise was higher ($P < 0.05$) in C than in HL (235 ± 41 *vs.* 167 ± 16 mmol ATP (kg wet wt)$^{-1}$), but when corrected for the work performed no difference was observed (Fig. 3). The aerobic energy production could account for 65% (C) and 64% (HL) of the total energy turnover.

Carbohydrate utilization

The muscle glycogen concentration decreased from 147·6 to 101·6 mmol (kg wet wt)$^{-1}$ in C and from 135·2 to 98·8 mmol (kg wet wt)$^{-1}$ in HL (Table 1). The mean rate of muscle glycogen breakdown was the same in C and HL (8·1 ± 1·2 *vs.* 8·2 ± 1·0 mmol (kg wet wt)$^{-1}$).

The muscle G-1-P concentration was the same in C and HL both before and at the end of exercises (Table 1). The pre-exercise values for G-6-P was the same for C and HL, but at the end of exercise G-6-P was 55% lower ($P < 0.05$) in C than in HL (Table 1). Similarly, F-6-P was 60% lower ($P < 0.05$) at the end of exercise in C compared with HL, with no difference prior to the exercise (Table 1).

The arterial glucose concentration prior to the exercise was the same in C and HL (5·23 ± 0·45 and 5·45 ± 0·86 mmol l^{-1}, respectively), and only minor changes occurred during the exercise, the values at exhaustion being 5·50 ± 0·52 and 5·19 ± 0·84 mmol l^{-1}, respectively. No net exchange of glucose was observed before and during the initial phase of the exhaustive exercise bouts. At 1·55 min in C a net release of glucose (0·40 ± 0·21 mmol min^{-1}; $P < 0.05$) was observed. During the rest of the exercise in C and in HL no net exchange of glucose occurred, except at exhaustion where a net uptake was found in both C and HL (0·75 ± 0·09 and 0·66 ± 0·19 mmol min^{-1}, respectively). The total net uptake of glucose during exercise was 0·64 ± 0·27 (C) and 0·48 ± 0·31 mmol (HL) (n.s.).

Muscle H^+ concentration

Muscle H^+ concentration before the exercise was the same in C and HL (corresponding pH: 7·17 *vs.* 7·10), but at the end of exercise muscle H^+ concentration was lower ($P < 0.05$) in C than in HL (corresponding pH: 6·82 *vs.* 6·65; Table 1).

Potassium exchange

Both the arterial and venous plasma potassium concentrations prior to the exercise were higher ($P < 0.05$) in C than in HL (Fig. 4). Arterial potassium concentration remained higher ($P < 0.05$) in C compared with HL during the initial phase of the exercise, but no differences in arterial and venous potassium concentrations between the two trials were observed during the later phase of the exercise and at exhaustion.

A net exchange of potassium was not observed prior to the exercise in either C or HL (Fig. 4; $P < 0.05$). During exercise the leg released potassium in both C and HL, and in the initial phase the release was higher ($P < 0.05$) in HL than in C. The total efflux of potassium during the exercise was the same in C and HL (6·7 ± 1·4 *vs.* 7·2 ± 0·9 mmol), but when 'normalized' for the difference in exercise time the release of potassium was less ($P < 0.05$) in C compared with HL (5·4 ± 1·3 *vs.* 7·0 ± 0·9 mmol).

Hormones

The plasma adrenaline concentration was the same in C and HL both before (0·25 ± 0·06 and 0·33 ± 0·08 ng ml^{-1}, respectively) and at the end of the exercise (0·47 ± 0·15 *vs.* 0·47 ± 0·08 ng ml^{-1}, respectively). On the other hand, the plasma noradrenaline concentration was lower ($P < 0.05$) in

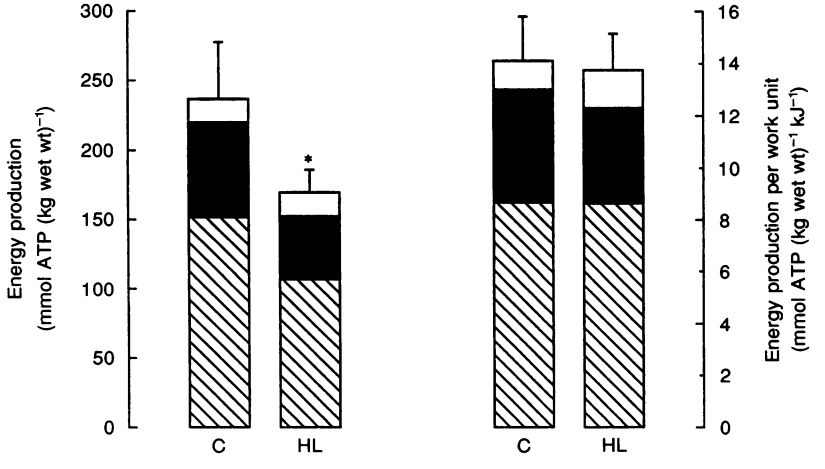

Figure 3. Energy production

Total energy production (left) and energy production per work unit (right) estimated from oxidation (▨), lactate production (■), CP and ATP utilization (□; $-\Delta CP - \Delta ATP + \Delta IMP$) during intense exhaustive exercise without (C) and with (HL) prior intense intermittent arm exercise. * Significantly different from C.

C than in HL both prior to (0·34 ± 0·07 and 0·70 ± 0·16 ng ml^{-1}, respectively) and at the end of exercise (1·00 ± 0·14 and 1·67 ± 0·34 ng ml^{-1}, respectively).

There was no difference in insulin between C and HL either before (11·0 ± 1·2 and 13·1 ± 0·9 μU ml^{-1}, respectively) or at the end of exercise (12·0 ± 1·0 and 11·3 ± 0·9 μU ml^{-1}, respectively).

DISCUSSION

The major findings of the present study were that muscle fatigue, defined as a failure to maintain the required power output (Edwards, 1981), occurred at a lower muscle pH, but at the same femoral blood potassium concentrations, when intense leg exercise was preceded by arm exercise. Furthermore, lowered muscle pH did not affect the rates of glycogen breakdown and lactate production.

The arm exercise in the HL experiment elevated leg muscle lactate concentration prior to the leg exercise as a result of the increased arterial lactate concentrations. Furthermore, when leg exercise was performed after the arm exercise the lactate, and probably the H^+, release was reduced, which probably led to the lowered muscle pH. It might seem strange that muscle pH at the end of exercise was lower in HL than in C as the muscle lactate concentration was the same in HL and in C and because a close association between muscle lactate and pH has been described (Sahlin, Harris, Nylind & Hultman, 1976). However, in addition to H^+ release through lactate–H^+ cotransport, H^+ may also be transported across the muscle membrane by other mechanisms such as Na^+–H^+ exchange (Juel, 1988*a*; Bangsbo, Graham, Johansen & Saltin, 1993). Thus, the finding of a lower muscle pH in HL compared with C, despite muscle lactate being the same, may suggest that the release of H^+ by the other mechanisms was less in HL compared with C, as it is unlikely that there were major differences in the muscle buffer capacity in the two conditions. Whatever caused the difference, the lower pH in HL experiments made it possible to study the effect of pH on the development of fatigue and metabolism during intense exercise.

The finding of a difference in muscle pH at exhaustion in the two conditions suggests that low pH is not the only factor causing fatigue during intense muscle contractions in the type of exercise used in the present study. This observation is in line with findings in a study in which subjects repeated intense exhaustive knee-extensor exercise after a 60 min recovery period (Bangsbo *et al.* 1992*b*). At the point of exhaustion the muscle lactate concentration in the second exercise bout was only 65% of the concentration at the end of the first exercise bout.

If muscle lactate and pH are not, or not always, causally linked with a decline in development of muscle tension, then what is the cause of fatigue? As the muscle CP concentrations were the same at the point of exhaustion, it may be suggested that low CP levels caused fatigue. However, it has been observed that CP decreases rapidly in the initial phase of exercise and that subjects are able to

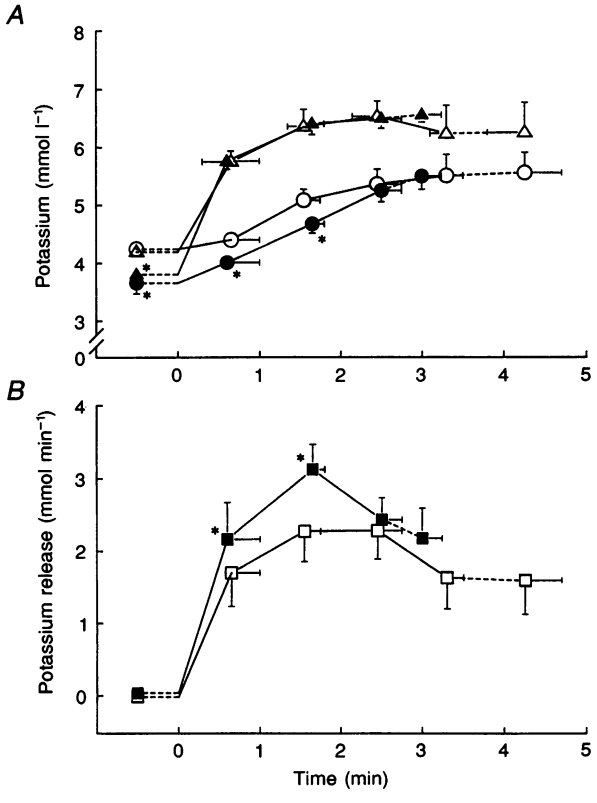

Figure 4. Blood potassium and potassium efflux
Arterial (circles) and venous (triangles) plasma potassium concentration (*A*) and potassium efflux (*B*) during intense exhaustive exercise without (C, open symbols) and with (HL, filled symbols) prior intense intermittent arm exercise. Values after the dashed lines mark measurements just before exhaustion. * Significantly different from C.

maintain the exercise intensity for several minutes even though the CP concentration remains low (Karlsson, 1971; Saltin, Gollnick, Eriksson & Piehl, 1972). Therefore, it is doubtful that low CP levels *per se* caused fatigue.

We and others have previously speculated that a progressive accumulation of potassium in the interstitium is implicated in the fatigue process (Sjøgaard, 1990; Bangsbo *et al.* 1992*a*, *b*). Prior to the exhaustive leg exercise both the arterial and femoral venous potassium concentrations were lower in HL than in C. On the other hand, during the exercise, the release of potassium was higher in HL than in C. This may have been due to an increased potassium conductance perhaps caused by the lower muscle pH in HL (Fink, Hase, Lüttgau & Wettwer, 1983; Davies, 1992). The larger release of potassium in HL led to the same arterial and venous potassium concentration at the point of exhaustion in HL and C, even though the exercise duration was shorter in HL. Furthermore, in both C and HL the potassium efflux to the blood was reduced at the end of exercise, possibly due to the increasing arterial potassium concentrations, suggesting that potassium in the interstitium accumulated progressively towards the end of exercise. The finding of the same arterial and venous potassium concentrations at exhaustion under the two conditions is in accordance with our previous observations in studies where intense exercise was repeated (Bangsbo *et al.* 1992*a*, *b*). It is also in agreement with the observation of the same arterial and venous potassium concentration at exhaustion, whether intense exercise was performed with or without β-blockade, although the exercise time was shorter with β-blockade (Hallen, Gullestad & Sejersted, 1994). The finding that the femoral arterial and venous potassium concentrations were the same in C and HL at exhaustion may indicate that fatigue occurs when a given potassium concentration is reached in the interstitium.

The mechanism behind the possible effect of potassium on the development of fatigue is unclear. It may be that the accumulating potassium stimulates sensory receptors of group III and IV nerve fibres leading to inhibition at the spinal level (Bigland-Ritchie & Woods, 1984; Bigland-Ritchie, Dawson, Johansson & Leppold, 1986; Garland & McComas, 1990). Another coupling between fatigue and potassium could be an inhibition of the propagation of the action potential due to ion disturbances over the sarcolemma and a possible block in its propagation into the t-tubules (Sjøgaard, 1990). The latter hypothesis is supported by the observation that rather small increments in extracellular potassium lead to a reduction in tension in subsequent contractions when isolated mouse muscles were stimulated (Juel, 1988*b*). It is noteworthy that even though the present results indicate that lowered pH *per se* does not cause fatigue, a decrease in pH may promote the development of fatigue during exercise by increasing the release of potassium from the muscle cell.

It cannot be excluded that a component of central (cortical) fatigue was involved in the inability to maintain the power output. However, there are several indications that the site for fatigue is local. The systemic effects of one-legged knee-extensor exercise are minor. Whole body oxygen uptake at the end of exercise in both conditions ($1{\cdot}96 \pm 0{\cdot}13$ (C) and $1{\cdot}94 \pm 0{\cdot}16$ l min^{-1} (HL)) was less than one-half of the subjects' maximum oxygen uptake and the concentrations of catecholamines were rather low. Furthermore, the subjects were highly motivated and the duration of the exhaustive exercises was similar to pre-experiments, indicating that the catheterization and biopsy procedures did not impair performance. In agreement with the suggestion that fatigue originated from the muscle are findings that electrical stimulation of a fatiguing quadriceps muscle did not lead to any increase in force development in well-motivated subjects (Bigland-Ritchie & Woods, 1984).

An elevated muscle H^+ concentration *in vitro* has been demonstrated to have an inhibitory effect on phosphorylase and phosphofructokinase (PFK), which are considered the key regulating enzymes of the glycogenolytic and glycolytic pathways, respectively (Danforth, 1965; Chasiotis *et al.* 1983; Amorena *et al.* 1990). However, in the present study the rate of glycogen breakdown was the same in C and HL, although muscle pH was significantly lower in HL. This suggests that changes in pH have little effect on glycogenolysis *in vivo*, which is in agreement with other findings using the knee-extensor model (Bangsbo *et al.* 1992*a*, *b*). Furthermore, the rate of pyruvate oxidation and lactate production was the same in C and HL. These findings imply that any effect of pH on glycolysis to a major extent is counteracted by other modulators *in vivo*. This suggestion is further supported by studies on the allosteric regulation of PFK which, within the physiological range of pH, have shown that the effect of pH is small (Dobson, Yamamoto & Hochachka, 1986; Spriet, Söderlund, Bergström & Hultman, 1987).

It has been speculated that low muscle pH could affect the energy turnover during intense exercise e.g. by lowering the energy release from ATP breakdown (Kawai, Guth, Winnikes, Haist & Ruegg, 1987; Cooke & Pate, 1990). However, in the present study the energy production expressed per unit of work was not different in the two situations (Fig. 3), although muscle pH was lower in HL. This finding suggests that muscle pH has little influence on the efficiency of the muscle contractions.

In summary, the present data suggest that decreasing muscle pH by about 0·2 units has no effect on muscle glycogenolysis, glycolysis and pyruvate oxidation during exercise. Furthermore, low muscle pH *per se* does not appear to cause fatigue during intense exercise. Instead, there may be a link between accumulation of interstitial potassium in muscle and the fatigue processes.

AMORENA, C. F., WILDING, T. J., MANCHESTER, J. K. & ROOS, A. (1990). Changes in intracellular pH caused by high K in normal and acidified frog muscle. *Journal of General Physiology* **96**, 959–972.

ANDERSEN, P. & SALTIN, B. (1985). Maximal perfusion of skeletal muscle in man. *Journal of Physiology* **366**, 233–249.

BANGSBO, J., AAGAARD, T., OLSEN, M., KIENS, B., TURCOTTE, L. P. & RICHTER, E. A. (1995). Lactate and H^+ uptake in inactive muscles during intense exercise in man. *Journal of Physiology* **488**, 219–229.

BANGSBO, J., GRAHAM, T. E., JOHANSEN, L. & SALTIN, B. (1993). Lactate and H^+ fluxes from skeletal muscles in man. *Journal of Physiology* **462**, 115–133.

BANGSBO, J., GRAHAM, T., JOHANSEN, L., STRANGE, S., CHRISTENSEN, C. & SALTIN, B. (1992*a*). Elevated muscle acidity and energy production during exhaustive exercise in humans. *American Journal of Physiology* **263**, R891–899.

BANGSBO, J., GRAHAM, T. E., KIENS, B. & SALTIN, B. (1992*b*). Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *Journal of Physiology* **451**, 205–227.

BIGLAND-RITCHIE, B., DAWSON, N. J., JOHANSSON, R. S. & LEPPOLD, O. C. J. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *Journal of Physiology* **379**, 451–459.

BIGLAND-RITCHIE, B. & WOODS, J. J. (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle and Nerve* **7**, 691–699.

BISCHOFF, E. (1863). Einige Gewichts-und Trocken-Bestimmungen der Organe des menschlichen Korpers. *Zeitschrift für Rationelle Medizin* III **20**, 75–118.

CHASIOTIS, D., HULTMAN, E. & SAHLIN, K. (1983). Acidotic depression of cyclic AMP accumulation and phosphorylase *b* to *a* transformation in skeletal muscle in man. *Journal of Physiology* **335**, 197–204.

COOKE, R. & PATE, E. (1990). The inhibition of muscle contraction by the products of ATP hydrolysis. In *Biochemistry of Exercise VII*, ed. TAYLOR, A. W., GOLLNICK, P. D., GREEN, H. J., IANUZZO, S. D., NOBBLE, E. G., METIVIER, G. & SUTTON, J. R., pp. 59–72. Human Kinetics Publications Inc., Champaign, IL, USA.

DANFORTH, W. H. (1965). Activation of glycolytic pathway in muscle. In *Control of Energy Metabolism*, ed. CHANCE, B., ESTRABROOK, B. W. & WILLIAMSON, J. R., pp. 287–297. Academic Press, New York.

DAVIES, N. W. (1992). ATP-dependent K^+ channels and other K^+ channels of muscle: how exercise may modulate their activity. In *Muscle Fatigue Mechanisms in Exercise and Training. Medicine of Sports Science* **34**, ed. MARCONNET, P., KOMI, P. V., SALTIN, B. & SEJERSTED, O. M., pp. 1–10. Karger, Basel.

DOBSON, G. P., YAMAMOTO, E. & HOCHACHKA, P. W. (1986). Phosphofructokinase control in muscle: nature and reversal of pH-dependent ATP inhibition. *American Journal of Physiology* **250**, R71–76.

DOUGLAS, A. R., JONES, N. J. & REED, J. W. (1988) Calculation of whole blood CO_2 content. *Journal of Applied Physiology* **65**, 473–477.

DRABKIN, D. L. & AUSTIN, J. H. (1935). Spectrophotometric studies II. Preparations from washed blood cells, nitric oxide hemoglobin and sulfhemoglobin. *Journal of Biological Chemistry* **122**, 51–65.

EDWARDS, R. H. T. (1981). Human muscle function and fatigue. In *Human Muscle Fatigue: Physiological Mechanisms*, ed. PORTER, R. & WHELAN, J., *Ciba Foundation Symposium*, vol. 82, pp. 1–18. Pitman Medical, London.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

FINK, R., HASE, S., LUTTGAU, H. CH. & WETTWER, E. (1983). The effect of cellular energy reserves and internal calcium ions on the potassium conductance in skeletal muscle of the frog. *Journal of Physiology* **336**, 211–228.

GARLAND, S. J. & MCCOMAS, A. J. (1990). Reflex inhibition of human soleus muscle during fatigue. *Journal of Physiology* **429**, 17–27.

HALLEN, J., GULLESTAD, L. & SEJERSTED, O. M. (1994). K^+ shifts of skeletal muscle during stepwise bicycle exercise with and without β-adrenoceptor blockade. *Journal of Physiology* **477**, 149–159.

HOGAN, M. C. & WELCH, H. G. (1984). Effect of varied lactate levels on bicycle ergometer performance. *Journal of Applied Physiology* **57**, 507–513.

HOLMGREN, A. & PERNOW, B. (1959). Spectrophotometric determination of oxygen saturation of blood in the determination of cardiac output. *Scandinavian Journal of Clinical Laboratory Investigation* **11**, 143–149.

JACOBS, I., HERMISTON, A. J. & SYMONS, J. D. (1993). Effects of prior exercise or ammonium chloride ingestion on muscular strength and endurance. *Medicine and Science in Sports Exercise* **25**, 809–814.

JONES, P. R. M. & PEARSON, J. (1969). Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *Journal of Physiology* **204**, 36*P*.

JUEL, C. (1988*a*). Intracellular pH recovery and lactate efflux in mouse soleus muscles stimulated *in vitro*: the involvement of sodium/proton exchange and a lactate carrier. *Acta Physiologica Scandinavica* **132**, 363–371.

JUEL, C. (1988*b*). The effect of β_2-adrenoceptor activation on ion-shifts and fatigue in mouse soleus muscles stimulated *in vitro*. *Acta Physiologica Scandinavica* **134**, 209–216.

KARLSSON, J. (1971). Lactate and phosphagen concentrations in working muscle of man. *Acta Physiologica Scandinavica*, suppl. 358, 7–72.

KARLSSON, J., BONDE-PETERSEN, F., HENRIKSSON, J. & KNUTTGEN, H. G. (1975). Effect of previous exercise with arms and legs on metabolism and performance in exhaustive exercise. *Journal of Applied Physiology* **38**, 763–767.

KAWAI, M., GUTH, K., WINNIKES, K., HAIST, C. & RUEGG, J. C. (1987). The effect of inorganic phosphate on ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. *Pflügers Archiv* **408**, 1–9.

LANNERGREN, J. & WESTERBLAD, H. (1991). Force decline due to fatigue and intracellular acidification in isolated fibres from mouse skeletal muscle. *Journal of Physiology* **434**, 307–322.

LOWRY, O. H. & PASSONNEAU, J. V. (1972). *A Flexible System of Enzymatic Analysis*. Academic Press, New York.

PARKHOUSE, W. S., MCKENZIE, D. C., HOCHACHAKA, P. W. & OVALLE, W. K. (1985). Buffer capacity of deproteinized human vastus lateralis muscle. *Journal of Applied Physiology* **58**, 14–17.

SAHLIN, K. (1986). Muscle fatigue and lactid acid accumulation. *Acta Physiologica Scandinavica* **128**, 83–91.

SAHLIN, K., HARRIS, R. C., NYLIND, B. & HULTMAN, E. (1976). Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflügers Archiv* **367**, 143–149.

SALTIN, B., GOLLNICK, P. D., ERIKSSON, B. O. & PIEHL, K. (1972). Metabolic and circulatory adjustments at onset of work. In *Onset of Exercise, Symposium*, ed. GILBERT, A. & GUILLE, L., pp. 63–76. Toulouse.

SJØGAARD, G. (1990). Exercise induced muscle fatigue: the significance of potassium. *Acta Physiologica Scandinavica* **140**, suppl. 593, 1–63.

SPRIET, L. L., LINDINGER, M. I., MCKELVIE, S., HEIGENHAUSER, G. J. F. & JONES, N. L. (1989). Muscle glycogenolysis and H^+ concentration during maximal intermittent cycling. *Journal of Applied Physiology* **66**, 8–13.

SPRIET, L. L., SDERLUND, K., BERGSTRM, M. & HULTMAN, E. (1987). Skeletal muscle glycogenolysis, glycolysis and pH during electrical stimulation in men. *Journal of Applied Physiology* **62**, 616–621.

TULLSON, P. C., WHITLOCK, D. M. & TERJUNG, R. L. (1990). Adenine nucleotide degradation in slow twitch red muscle. *American Journal of Physiology* **258**, C258–265.

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