Muscle oxygen uptake and energy turnover during dynamic exercise at different contraction frequencies in humans

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1. It has been established that pulmonary oxygen uptake is greater during cycle exercise in humans at high compared to low contraction frequencies. However, it is unclear whether this is due to more work being performed at the high frequencies and whether the energy turnover of the working muscles is higher. The present study tested the hypothesis that human skeletal muscle oxygen uptake and energy turnover are elevated during exercise at high compared to low contraction frequency when the total power output is the same.

2. Seven subjects performed single-leg dynamic knee-extensor exercise for 10 min at contraction frequencies of 60 and 100 r.p.m. where the total power output (comprising the sum of external and internal power output) was matched between frequencies (54 ± 5 *vs.* 56 ± 5 W; mean ± S.E.M.). Muscle oxygen uptake was determined from measurements of thigh blood flow and femoral arterial − venous differences for oxygen content (a–v O_2 diff). Anaerobic energy turnover was estimated from measurements of lactate release and muscle lactate accumulation as well as muscle ATP and phosphocreatine (PCr) utilisation based on analysis of muscle biopsies obtained before and after each exercise bout.

3. Whilst a–v O_2 diff was the same between contraction frequencies during exercise, thigh blood flow was higher ($P < 0.05$) at 100 compared to 60 r.p.m. Thus, muscle $\dot{V}_{O_2}$ was higher ($P < 0.05$) during exercise at 100 r.p.m. Muscle $\dot{V}_{O_2}$ increased ($P < 0.05$) by 0.06 ± 0.03 (12%) and 0.09 ± 0.03 l min^{-1} (14%) from the third minute to the end of exercise at 60 and 100 r.p.m., respectively, but there was no difference between the two frequencies.

4. Muscle PCr decreased by 8.1 ± 1.7 and 9.1 ± 2.0 mmol (kg wet wt)$^{-1}$, and muscle lactate increased to 6.8 ± 2.1 and 9.8 ± 2.5 mmol (kg wet wt)$^{-1}$ during exercise at 60 and 100 r.p.m., respectively. The total release of lactate during exercise was 48.7 ± 8.8 and 64.3 ± 10.6 mmol at 60 and 100 r.p.m. (not significant, NS). The total anaerobic ATP production was 47 ± 8 and 61 ± 12 mmol kg^{-1}, respectively (NS).

5. Muscle temperature increased ($P < 0.05$) from 35.8 ± 0.3 to 38.2 ± 0.2°C at 60 r.p.m. and from 35.9 ± 0.3 to 38.4 ± 0.3°C at 100 r.p.m. Between 1 and 7 min muscle temperature was higher ($P < 0.05$) at 100 compared to 60 r.p.m.

6. The estimated mean rate of energy turnover during exercise was higher ($P < 0.05$) at 100 compared to 60 r.p.m. (238 ± 16 *vs.* 194 ± 11 J s^{-1}). Thus, mechanical efficiency was lower ($P < 0.05$) at 100 r.p.m. (24 ± 2%) compared to 60 r.p.m. (28 ± 3%). Correspondingly, efficiency expressed as work per mol ATP was lower ($P < 0.05$) at 100 than at 60 r.p.m. (22.5 ± 2.1 *vs.* 26.5 ± 2.5 J (mmol ATP)$^{-1}$).

7. The present study showed that muscle oxygen uptake and energy turnover are elevated during dynamic contractions at a frequency of 100 compared with 60 r.p.m. It was also observed that muscle oxygen uptake increased as exercise progressed in a manner that was not solely related to the increase in muscle temperature and lactate accumulation.

The nature of the contractile and metabolic properties of skeletal muscle suggests that the speed of shortening will influence energy turnover during contractions. Indeed, it has been observed *in vitro* using isolated muscles and single muscle fibres that energy turnover expressed in relation to the work performed during contraction (i.e. efficiency) varies with shortening velocity (Kushmerick & Davies, 1969; Lodder *et al.* 1991; Reggiani *et al.* 1997; He *et al.* 2000). There has been considerable effort put into studying energy turnover in humans during exercise at different speeds of shortening, i.e. contraction frequencies. Traditionally, whole body exercise such as cycling has been used in which pulmonary oxygen uptake has been shown to increase as contraction (pedal) frequency increases (e.g. Gaesser & Brooks, 1975; Coast & Welch, 1985). However, it is not clear whether the differences in pulmonary oxygen uptake observed reflect a difference in the oxygen uptake of the contracting muscles. To overcome this the single-leg dynamic knee-extensor exercise model can be used since the contractions are confined to the quadriceps muscle group (Andersen *et al.* 1985; Ray & Dudley, 1998; Richardson *et al.* 1998). Muscle oxygen uptake as well as anaerobic energy turnover can be accurately determined (Bangsbo *et al.* 1990) to provide a full picture of total energy turnover during exercise.

The observation of higher pulmonary oxygen uptake at higher pedal frequencies during cycle exercise may have been due to the subjects performing more work, since the total work was not determined. Thus, one problem with quantifying muscle oxygen uptake and energy turnover at different contraction frequencies relates to ensuring that the mechanical work remains the same across the contraction frequencies examined, i.e. the so-called 'internal work' has to be considered. Internal work refers to work that is performed to overcome gravitational and inertial forces related to the movement of the body segments with respect to the body centre of mass (Fenn, 1930; Cavagna & Kaneko, 1977). It is clear that internal work is greater at a higher contraction frequency during different forms of locomotion (e.g. Wells *et al.* 1986; Minetti *et al.* 1995). We have recently addressed this problem using the dynamic knee-extensor exercise model and developed a method to quantify the total mechanical power output (i.e. external and internal power) during exercise at different contraction frequencies (Ferguson *et al.* 2000). Moreover, it was possible to match the total power output generated during exercise at different contraction frequencies by varying the external resistance of the ergometer (Ferguson *et al.* 2000) to allow the determination of muscle oxygen uptake, energy turnover and mechanical efficiency at identical levels of total power output.

A characteristic response during constant load exercise is a slowly developing component of pulmonary oxygen uptake, which has been termed the 'slow component' of oxygen uptake (Whipp & Wasserman, 1972). This slow component may in part be due to a progressive increase in oxygen uptake of the contracting muscles and may represent a change in the efficiency of muscle contraction (Poole *et al.* 1991). However, it is as yet unclear what may cause the slow component. It has been suggested that an increase in muscle temperature and muscle lactate concentration leading to a lowered muscle pH may be responsible for the slow rise in oxygen uptake in humans (see Gaesser & Poole, 1996). By comparing the slow component of muscle oxygen uptake at different contraction frequencies and relating any differences to changes in metabolic and mechanical variables, the importance of these factors may be evaluated.

Thus, the aim of the present study was to test the hypothesis that muscle oxygen uptake and total energy turnover are higher, and thus mechanical efficiency of human skeletal muscle is lower, during dynamic exercise at a high compared to low contraction frequency. A further aim was to examine whether muscle oxygen uptake increases as exercise progressed at a constant intensity, and to what extent such a change is related to muscle temperature and muscle lactate accumulation and production.

METHODS

Subjects

Seven healthy male subjects volunteered to participate in the investigation. All the subjects were physically active but none were specifically trained. Mean (± S.E.M.) age, height and body mass was 23 ± 1 years, 179.0 ± 2.5 cm, 72.4 ± 3.1 kg, respectively. The subjects were fully informed of the risks and discomfort associated with the experiment before providing written consent. The study was approved by the Copenhagen Ethics Committee and conformed with the Declaration of Helsinki.

Experimental design

Single-leg dynamic knee-extension exercise (Andersen *et al.* 1985) was performed in the supine position. All the subjects were fully habituated to the exercise procedures involved. Exercise was performed at contraction frequencies of 60 and 100 r.p.m. Prior to the main experiment subjects undertook detailed preliminary testing in order to ensure the same total power output was performed between the two contraction frequencies. Total power output comprises the external power delivered to the ergometer in addition to the internal power generated to overcome inertial and gravitational forces related to movement of the lower limb. The details of this method have been described previously (Ferguson *et al.* 2000). Briefly, total power output was determined during exercise at increasing external power outputs at both 60 and 100 r.p.m. From the relationship between external and calculated total power output at 100 r.p.m. an external power output was estimated that would result in the same total power output as that required at 60 r.p.m. The external power outputs were 41 ± 4 and 27 ± 5 W at 60 and 100 r.p.m., respectively.

During the main experiment the exercising thigh was covered with a cuff prior to each exercise bout through which warm water (~37 °C) was perfused. This was in order to stabilise the skin temperature without causing any increase in resting muscle temperature (~36 °C). The procedure normally took 10–15 min after which the exercise protocol commenced with the thigh remaining covered during the exercise.

Femoral venous temperature was measured and multiple blood samples were collected frequently during the initial phase (2 min) of the 10 min exercise bout (Fig. 1*B*), which prevented accurate blood flow measurements from being made. Therefore, after a sufficient recovery period (~60 min) a second 2 min exercise bout was performed where blood flow was measured (Fig. 1*A*). It has been shown that the rate in rise of blood flow is similar with repeated bouts of exercise separated by around 60 min (Bangsbo *et al.* 1992, 2000).

Experimental procedures

The subjects arrived at the laboratory at 8 am after a standardised breakfast (consisting of fruit juice and cereal) and rested in the supine position. During this time a catheter to collect arterial blood samples was placed, under local anaesthesia, into the femoral artery of the non-exercising (left) leg with the tip positioned approximately 2 cm proximal to the inguinal ligament. A second catheter was inserted in the femoral artery of the experimental (right) leg through which a thermistor (Edslab, T.D. Probe, 94-030-2.5F, Baxter A/S, Allerod, Denmark) was placed for measurement of blood temperature. A third catheter was placed in the femoral vein of the experimental leg (right) with the tip positioned 2 cm distal to the inguinal ligament. A thermistor for measurement of blood temperature was advanced approximately 8 cm beyond the tip of the venous catheter also for measurement of thigh blood flow.

For measurements of muscle temperature the technique described by González-Alonso *et al.* (2000) was used. Briefly, five flexible thermistors (Edslab, T.D. Probe, 94-030-2.5F) were inserted 3–4 cm into the muscles of the quadriceps. The thermistors were inserted through a flexible venflon cannula (18G) and advanced ~0.5 cm beyond the end of the cannula into the muscle. Two thermistors were positioned in the vastus lateralis (proximal and distal), two in the rectus femoris (proximal and distal) and one in the vastus medialis (distal). A thermistor was also positioned in a hamstring muscle (medial biceps femoris). The probes were inserted at different angles (30, 45 and 60 deg) with respect to the length direction of the muscle fibres. This ensured varying depths of muscle temperature measurement (2–4 cm) and minimised movement of the thermistors and discomfort to the subjects. Five thermistors (Ellab, Copenhagen, Denmark) were placed on the skin next to the insertion points of muscle thermistors and all were secured with micropore tape.

Experimental protocol

Following preparation the subjects rested for ~30 min. After this time the thigh cuff was applied and after ~15 min when skin temperature had equilibrated with muscle temperature resting blood flow was measured, arterial and venous blood samples taken, and a resting muscle biopsy was obtained. After this the first 10 min exercise bout commenced (Fig. 1*A*) at either at 60 or 100 r.p.m. (the order of which was randomised). For 15 s prior to the onset of exercise

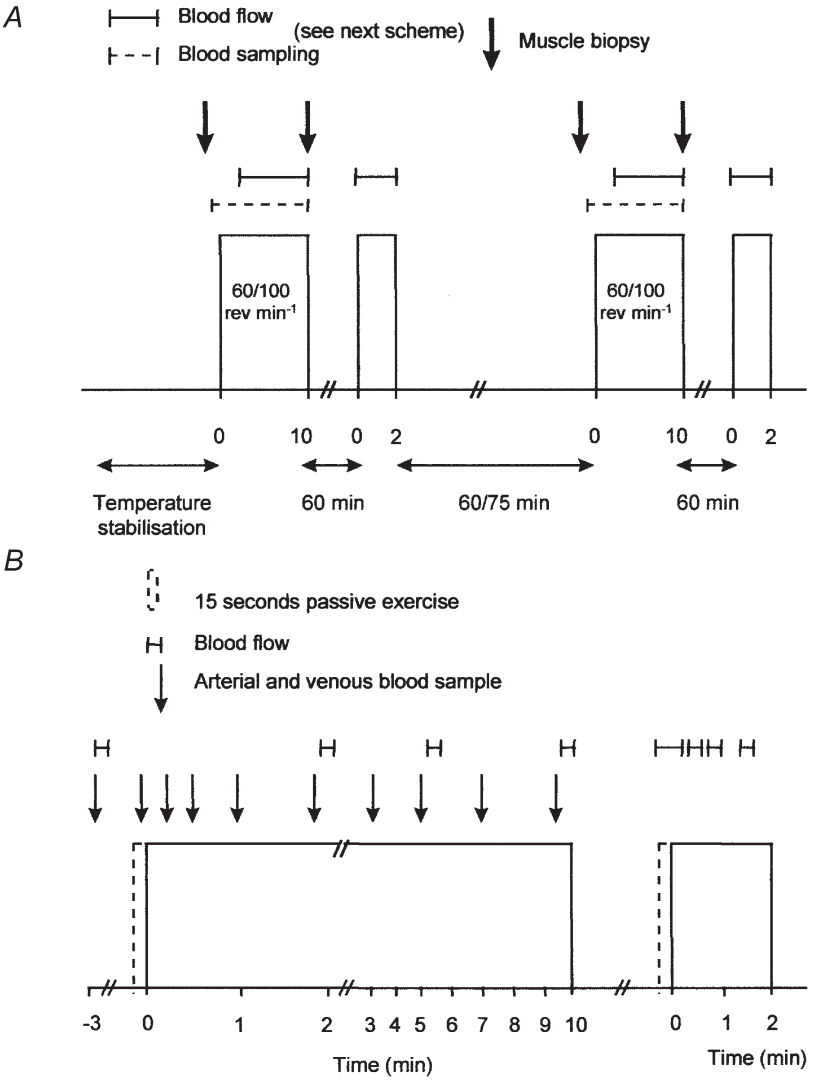

Figure 1. Schematic representation of the experimental protocol

A, the overall experimental protocol. *B*, time of blood sampling and blood flow measurements during each exercise bout.

the leg was passively moved in order to accelerate the ergometer flywheel and ensure a constant power output from the onset of exercise. Blood was drawn frequently from the femoral artery and vein during the passive exercise and 15 s after the constant load exercise had commenced. Further samples were taken at approximately 30 s, 1, 2, 3, 5, 7 and 9.5 min of exercise (Fig 1*A*). Thigh blood flow was measured immediately after the venous blood sample was collected at 2, 5, 7 and 9.5 min (Fig. 1*B*). After 10 min the leg was rapidly stopped (< 2 s) and a muscle biopsy was obtained within 5 s. The thigh cuff was removed and the thigh was allowed to cool down, which took at least 60 min, facilitating a sufficient recovery period. When the thigh had cooled to pre-experimental temperatures the cuff was reapplied and after 15 min the 2 min exercise bout commenced, at the same contraction frequency as previously performed. Again, the leg was moved passively for 15 s prior to exercise. Thigh blood flow was measured during the transition between the passive leg movement and the onset of exercise and at 15, 30, 60 and 90 s of exercise (Fig. 1*B*). After this second bout of exercise the thigh cuff was again removed to allow the thigh to cool down. After another 60–75 min rest period the entire protocol was repeated with exercise performed at the reciprocal contraction frequency.

Measurements

Muscle mass. The mass of the quadriceps femoris muscle group was estimated using measurements of thigh length, multiple circumferences of the thigh- and skin-fold thickness (Jones & Pearson, 1969), and corrected based on a comparison between anthropometric measurements and MRI-scan determinations (ratio 1: 0.8; Hellsten *et al.* 1999). The mean knee-extensor mass of the experimental leg was 2.26 ± 0.06 kg.

Total power output. Total power output was measured as previously described (Ferguson *et al.* 2000). Flexible electrogoniometers (type XM180, Penny & Gilles, Biometrics, Gwent, UK) were positioned to measure the knee joint angle and the angle between the connecting bar of the ergometer and the lower limb. Instantaneous muscle power was calculated as the product of knee joint angular velocity (expressed in rads per second) and total muscle moment (expressed in newton metres). Total muscle moment was calculated as $M_{\text{total}} = M_{\text{ergometer}} + M_{\text{gravity}} + M_{\text{inertia}} \times M_{\text{ergometer}}$ and was the muscle moment generated to overcome the resistance of the ergometer flywheel system. M_{gravity} was the muscle moment produced to overcome the force of gravity acting on the lower limb. M_{inertia} was the muscle inertial moment depending on acceleration and deceleration of the lower limb (Ferguson *et al.* 2000). Total power output was determined for the extension phase of each duty cycle by integration of the instantaneous power output curve and subsequent division by the time period of integration. Five data sweeps, each of 10 s duration, were obtained and analysed for each trial. Data from all five sweeps were averaged to obtain the mean total power.

Thigh blood flow. Femoral venous blood flow (i.e. thigh blood flow) was measured by the constant infusion thermodilution technique (Andersen & Saltin, 1985) and modified by Gonzalez-Alonzo *et al.* (2000). Briefly, venous and infusate temperatures were measured continuously before and during ice-cold saline infusion (10–15 s) at a rate of 120 ml min^{-1}. This achieved a drop in venous blood temperature of ~1–2 °C. Resting blood flow measurements were made with an infusion rate of ~45 ml min^{-1} for 30–45 s. Venous temperature was measured with the thermistor positioned through the venous catheter. Infusate temperature (0–4 °C) was measured at the site of entry to the catheter. An occlusion cuff placed below the knee was inflated (250 mmHg) 30 s prior to the exercise and remained inflated throughout exercise in order to avoid contribution of blood from the lower leg.

Blood samples. All arterial and venous blood samples were immediately analysed for P_{O_2}, O_2 saturation and haemoglobin (ABL510, Radiometer, Copenhagen, Denmark) from which O_2 content was calculated. For the determination of blood lactate and glucose (Yellow Spring Instruments, Yellow Springs, OH, USA) 200 μl of whole blood was haemolysed within 10 s of sampling by adding to 200 μl of buffer (YSI; 0.5% Triton X-100).

Muscle biopsies. Muscle samples, taken from the medial part of the vastus lateralis using the needle biopsy technique (Bergstrom, 1962) with suction, were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analysis. The frozen samples were weighed before and after freeze drying to determine water content. The freeze-dried samples were dissected free of blood and connective tissue and prepared for metabolite analysis. Metabolites were extracted in a solution of 0.6 M perchloric acid (PCA) and 1 mM EDTA, neutralised to pH 7.0 with 2 M $KHCO_3$ and stored at −80 °C. Phosphocreatine and lactate were analysed fluorimetrically (Lowry & Passonneau, 1972). ATP was extracted in 1 M PCA, neutralised to pH 8.0 with 2 M KOH and stored at −80 °C before analysis using reverse phase high performance liquid chromatography as described by (Tullson *et al.* 1990). Glycogen was extracted in 1 M HCl and assayed fluorimetrically (Lowry & Passonneau, 1972).

Data sampling

Arterial and venous blood temperature, saline infusate temperatures as well as strain gauge and goniometer signals were recorded at 400 Hz analog-to-digital sampling rate (MacLab 8s data acquisition system, Chart v3.3 software, ADInstruments, Sydney, Australia) onto the hard drive of a computer (Apple Macintosh Performa 5200). Muscle thermistors were connected via a custom-made interface and external A/D converter to an IBM computer (Gonzalez-Alonzo *et al.* 2000). Data were displayed on-line and stored on a hard disk using LabView v4.0 software (National Instruments, Austin, TX, USA). Muscle temperature data were averaged per 5 s. Skin thermistors were recorded via an eight-channel temperature monitor (Ellab CTF 9008 Precision thermometer, Ellab, Copenhagen, Denmark) interfaced with an IBM computer (Gonzalez-Alonzo *et al.* 2000). Data were displayed on-line and stored on a hard-disk every 15 s (PCLink 92; Ellab, Copenhagen, Denmark).

Calculations

Thigh oxygen uptake ($\dot{V}_{O_2}$) and lactate release were calculated by multiplying blood flow with arterial − venous O_2 difference and venous − arterial lactate difference, respectively. For the duration of exercise a continuous blood flow curve was constructed for each subject by linear interpolation of the measured blood flow data points to obtain time-matched values of blood flow with the blood variables. The total oxygen uptake and lactate release during the 10 min exercise was determined as:

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

where $t = 0$ is the start of exercise and x represents the end of exercise, and $f(t)$ the uptake/release of oxygen/lactate at a given time (t). Thus, uptake/release was determined as the area under the $f(t)$–time curve. The curves were produced on the assumption that there was a linear relationship with time in the interval between two consecutive values.

Thigh $\dot{V}_{O_2}$ was converted to mmol ATP using a P/O ratio of 2.5 mol ATP/mol O (Hinkle *et al.* 1991) and aerobic energy turnover was calculated using a caloric value of 21.2 kJ (l O_2)$^{-1}$. Anaerobic ATP production by the quadriceps for the total exercise duration was calculated as:

$$-\Delta\text{ATP} - \Delta\text{PCr} + 1.5\,\Delta\text{muscle lactate} + 1.5\,\text{lactate release} + \text{others}$$

'Others' represent ATP production related to accumulation of pyruvate assumed to be 1/30 th of accumulated muscle lactate (Spriet *et al.* 1987), lactate uptake by inactive tissues of the exercising leg (Bangsbo *et al.* 1995) and accumulation of glycolytic intermediates (Spriet *et al.* 1987). Anaerobic energy turnover was determined from the net change in reactant levels and average values of energy produced in each of the reactions determined *in vitro*. ΔH (molar enthalpy change) values of 55 and 67 kJ mol^{-1} of ATP produced were used for the net creatine phosphate breakdown and glycolysis leading to lactate formation, respectively (Walsh & Woledge, 1970; Curtin & Woledge, 1978). Muscle ATP production (mmol ATP) and the mean rate of energy turnover (J s^{-1}) was determined as the sum of aerobic and anaerobic ATP production and energy turnover, respectively. Mechanical efficiency was defined as the ratio between total mechanical power output (J s^{-1}) and total energy turnover (J s^{-1}). Efficiency was also calculated as the ratio between the total work performed (J) and the total ATP production (mmol ATP kg^{-1}).

Statistical analyses

Data were analysed by either paired t tests or two-way (contraction frequency and time) analysis of variance (ANOVA) with repeated measures, where appropriate. When a significant effect was observed differences were located with *post hoc* paired t tests. Significance was accepted at $P < 0.05$ and data are presented as means ± S.E.M.

RESULTS

Power output

The external power delivered to the ergometer at 60 r.p.m. was 41 ± 4 W and internal power output was 13 ± 4 W. In order to match the total power output of the two contraction frequencies the external power setting at 100 r.p.m. was 27 ± 5 W, resulting in a total power output of 56 ± 5 W, which was similar to the power output at 60 r.p.m. (54 ± 5 W).

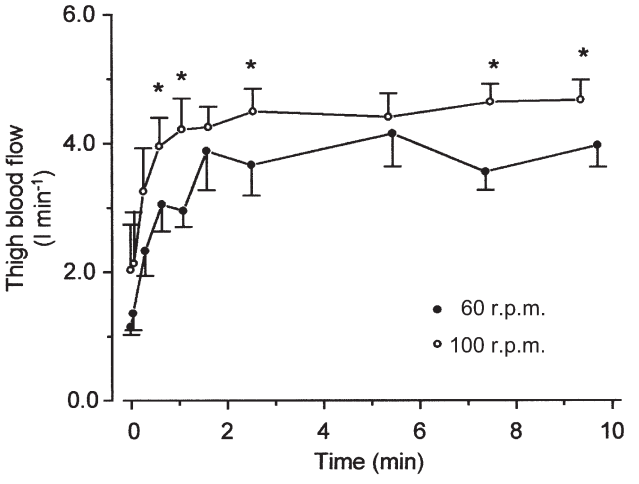

Figure 2. Thigh blood flow during knee-extensor exercise at contraction frequencies of 60 (●) and 100 (○) r.p.m.

Mean ± S.E.M. ($n = 7$). Time 0 represents the start of exercise at a constant external power output that was preceded by 15 s passive leg movement. Significant difference between 60 and 100 r.p.m. is indicated by * ($P < 0.05$).

Thigh blood flow

At the end of the 15 s passive movement, thigh blood flow was 1.15 ± 0.12 l min^{-1} at 60 r.p.m. and 2.03 ± 0.71 l min^{-1} at 100 r.p.m. (Fig. 2). After 45 s of exercise, blood flow was higher ($P < 0.05$) at 100 compared to 60 r.p.m.

Thigh oxygen uptake

Immediately prior to, and during, exercise arterial − venous O_2 difference (a–v O_2 diff) was the same at the two frequencies (Fig. 3*A*). Before exercise thigh oxygen uptake ($\dot{V}_{O_2}$) was similar at the two contraction frequencies but after 45 s thigh $\dot{V}_{O_2}$ was higher ($P < 0.05$) at 100 compared to 60 r.p.m. (Fig. 3*B*). There was a progressive increase ($P < 0.05$) in thigh $\dot{V}_{O_2}$ throughout exercise at both contraction frequencies. From the third minute to the end of exercise, thigh $\dot{V}_{O_2}$ increased ($P < 0.05$) by 0.06 ± 0.03 (12 %) and 0.09 ± 0.03 l min^{-1} (14 %) at 60 and 100 r.p.m., respectively (not significant (NS) between contraction

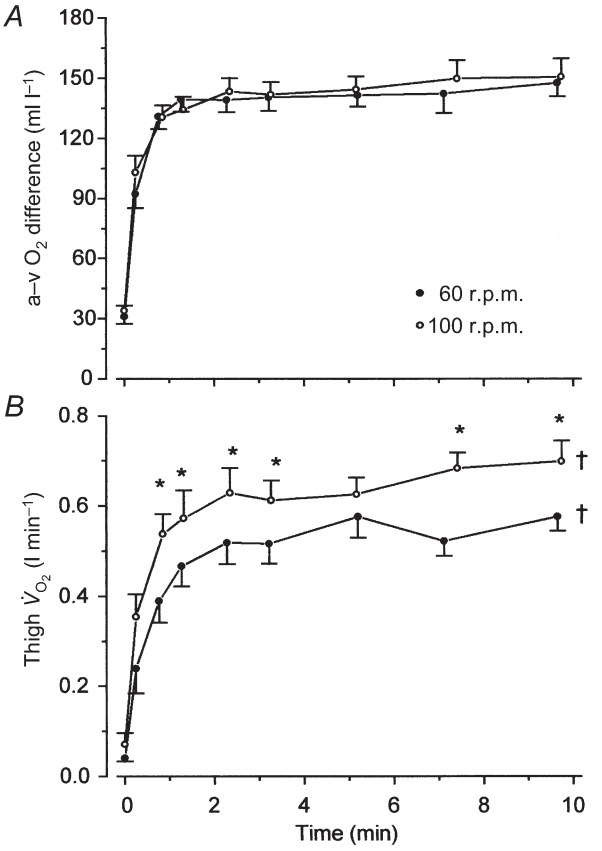

Figure 3. Thigh a–v O_2 difference (*A*) and oxygen uptake (*B*) during knee-extensor exercise at contraction frequencies of 60 (●) and 100 (○) r.p.m.

Mean ± S.E.M. ($n = 7$). Time 0 represents the start of exercise at a constant external power output that was preceded by 15 s passive leg movement. Significant difference between 60 and 100 r.p.m. is indicated by * ($P < 0.05$). † ($P < 0.05$) indicates significant difference between values at 3 min and end-exercise.

Table 1. Concentration of muscle metabolites and percentage water (% H_2O) at rest and following dynamic knee extensor exercise at 60 and 100 r.p.m.

	60 r.p.m.		100 r.p.m.	
Time (min)	0	10	0	10
ATP	4.5 ± 0.1	4.1 ± 0.3	5.0 ± 0.2	4.3 ± 0.3
PCr	18.4 ± 0.9	10.3 ± 1.5*	19.7 ± 0.9	10.6 ± 1.6*
Lactate	1.5 ± 0.3	6.8 ± 2.1*	1.2 ± 0.2	9.8 ± 2.5*
Glycogen	107 ± 19	81 ± 13*	105 ± 16	84 ± 15*
%H_2O	77.4 ± 0.8	78.9 ± 0.7	76.8 ± 0.7	78.1 ± 0.6

Values are in mmol (kg wet wt)$^{-1}$, (mean ± S.E.M.; $n = 7$, except for ATP where $n = 4$). Significant difference between 0 and 10 min denoted by * ($P < 0.05$).

frequencies). Total thigh V_{O_2} was greater ($P < 0.05$) at 100 compared to 60 r.p.m. (5.9 ± 0.4 *vs.* 4.8 ± 0.3 l).

Thigh lactate release

Immediately before exercise no venous − arterial lactate difference was observed and during exercise it increased ($P < 0.05$) to similar levels at 60 and 100 r.p.m. (Fig. 4*A*). Similarly, no net exchange of lactate was observed prior to exercise and the release of lactate during exercise was not different between the contraction frequencies (Fig. 4*B*). The total release of lactate during the 10 min of exercise was 48.7 ± 8.8 and 64.3 ± 10.6 mmol at 60 and 100 r.p.m., respectively (NS).

Muscle metabolites

There was no significant decrease in the muscle content of ATP during exercise at each contraction frequency (Table 1). Phosphocreatine (PCr) declined by approximately 45% at both frequencies (Table 1). Muscle lactate at the end of exercise was 6.8 ± 2.1 and 9.8 ± 2.5 mmol (kg wet wt)$^{-1}$ at 60 and 100 r.p.m., respectively (NS; Table 1). Muscle glycogen was the same prior to exercise and declined to a similar extent at each contraction frequency (Table 1). The percentage of muscle water was

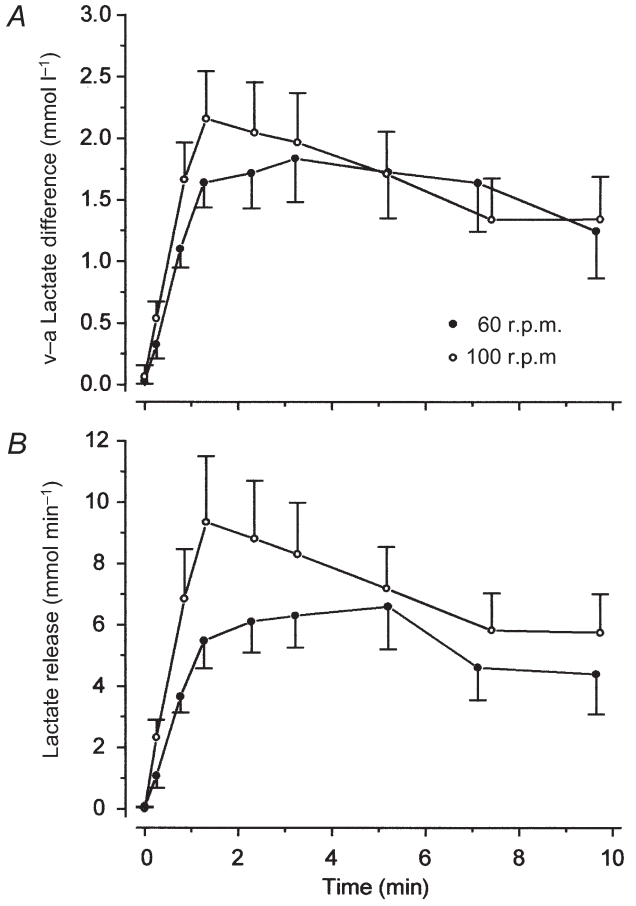

Figure 4. Thigh v–a lactate difference (*A*) and lactate release (*B*) during knee-extensor exercise at contraction frequencies of 60 (●) and 100 (○) r.p.m.

Mean ± S.E.M. ($n = 7$). Time 0 represents the start of exercise at a constant external power output that was preceded by 15 s passive leg movement.

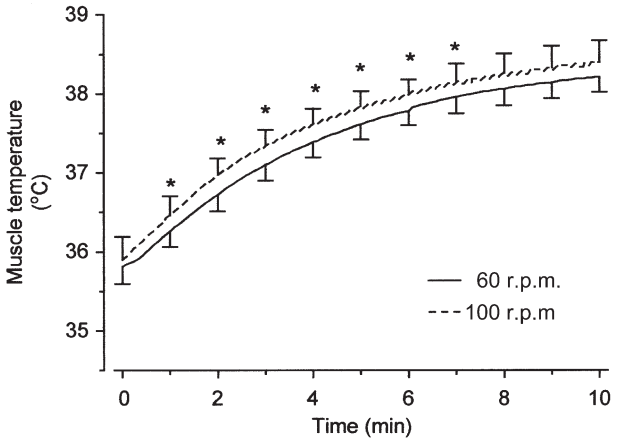

Figure 5. Quadriceps muscle temperature during knee-extensor exercise at contraction frequencies of 60 (continuous line) and 100 (dashed line) r.p.m.

Mean ± S.E.M. ($n = 7$). Time 0 represents the start of exercise at a constant external power output that was preceded by 15 s passive leg movement. Significant difference between 60 and 100 r.p.m. is indicated by * ($P < 0.05$).

the same at rest and did not change following exercise at either contraction frequency (Table 1).

Muscle ATP production, energy turnover and mechanical efficiency

The total net muscle lactate production (net lactate release + net lactate accumulation; 25.7 ± 4.8 *vs.* 35.9 ± 7.0 mmol kg^{-1}) and total anaerobic ATP production (47 ± 8 *vs.* 61 ± 12 mmol kg^{-1}) were not significantly different between 60 and 100 r.p.m., respectively. The total ATP production, including the aerobic ATP turnover, was higher ($P < 0.05$) at 100 r.p.m. than at 60 r.p.m. (621 ± 55 *vs.* 507 ± 41 mmol kg^{-1}, respectively). The mean rate of energy turnover for the 10 min of exercise was 194 ± 11 J s^{-1} at 60 r.p.m. which was lower ($P < 0.05$) than at 100 r.p.m. (238 ± 16 J s^{-1}). Thus, the average amount of work per mmol ATP production was 26.5 ± 2.5 and 22.5 ± 2.1 J (mmol ATP)$^{-1}$ at 60 and 100 r.p.m., respectively ($P < 0.05$). Mechanical efficiency was lower ($P < 0.05$) at 100 than at 60 r.p.m. (24 ± 2 *vs.* 28 ± 3 %).

Muscle and blood temperatures

Prior to the start of exercise the average temperature of the quadriceps muscle group was 35.8 ± 0.3 and 35.9 ± 0.3 °C at 60 and 100 r.p.m., respectively (Fig. 5) and at the end of exercise it reached 38.2 ± 0.2 and 38.4 ± 0.3 °C at 60 and 100 r.p.m., respectively. After 1 min and up to 7 min muscle temperature was higher ($P < 0.05$) during exercise at 100 r.p.m. compared to 60 r.p.m. Hamstrings muscle temperature prior to the onset of exercise was 36.1 ± 0.2 °C at both contraction frequencies and it increased steadily ($P < 0.05$) throughout exercise at both frequencies to 37.3 ± 0.4 °C at 60 r.p.m. and 37.9 ± 0.5 °C at 100 r.p.m. (NS). Arterial blood temperature increased from 37.1 ± 0.0 °C at 60 r.p.m. and 37.2 ± 0.0 °C at 100 r.p.m. to 37.5 ± 0.1 and 37.6 ± 0.0 °C, respectively. Venous blood temperature increased from 36.7 ± 0.1 to 37.7 ± 0.1 °C at 60 r.p.m. and from 36.8 ± 0.1 to 37.9 ± 0.1 °C at 100 r.p.m.

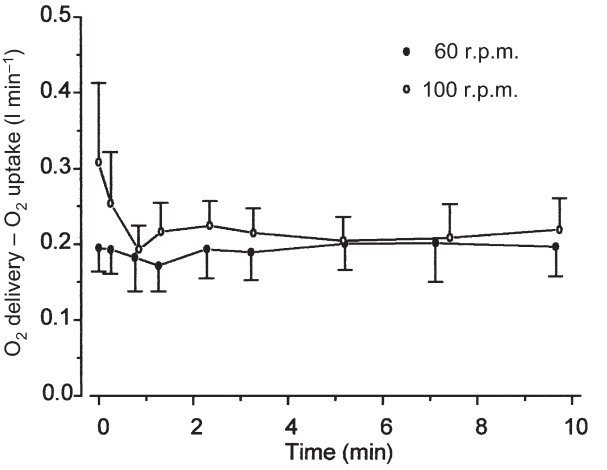

Figure 6. Difference between thigh oxygen delivery (thigh blood flow × arterial O_2 content) and thigh oxygen uptake (thigh blood flow × a–v O_2 diff) during knee-extensor exercise at contraction frequencies of 60 (●) and 100 (○) r.p.m.

Mean ± S.E.M. ($n = 7$). Time 0 represents the start of exercise at a constant external power output that was preceded by 15 s passive leg movement.

DISCUSSION

The present study supports the hypothesis that muscle oxygen uptake and the rate of energy turnover is greater when exercise is performed at a high compared to a low contraction frequency at the same power output. Thus, mechanical efficiency was lower at a high compared to

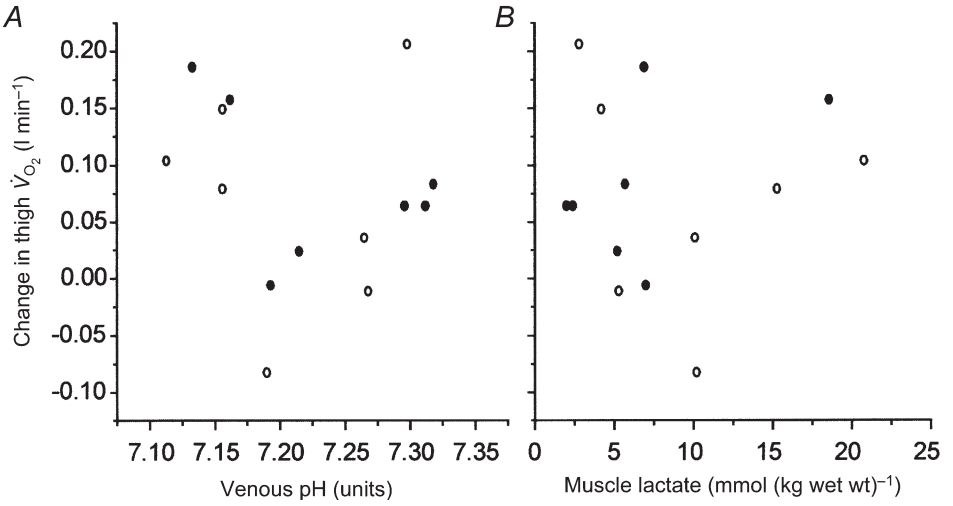

Figure 7

Relationships between the change in thigh oxygen uptake between 3 min and the end of exercise and femoral venous pH (A) and muscle lactate (B) during knee-extensor exercise at contraction frequencies of 60 (●) and 100 (○) r.p.m. Mean ± S.E.M. ($n = 7$).

low contraction frequency. In addition, thigh blood flow was higher during exercise at the high contraction frequency. Furthermore, throughout exercise at both contraction frequencies, muscle oxygen uptake increased progressively and by a similar magnitude.

Mechanical power output at different contraction velocities

A critical issue when studying mechanical efficiency in humans is to determine the total mechanical power produced, i.e. the power output that incorporates both external and internal power, in particular when different contraction frequencies are to be compared. Since internal power is greater at higher contraction frequencies the external power delivered to the ergometer has to be adjusted accordingly. The methodology used in the present study has been addressed carefully in a separate article (Ferguson *et al.* 2000). Briefly, the determination of internal power was composed of calculations of the moments produced to overcome inertial and gravitational forces related to movement of the lower limb, as first recognised by Fenn (1930) and later by Cavagna & Kaneko (1977). As expected from the difference in acceleration between the two contraction frequencies, the inertial properties associated with movement of the limb were greater at 100 compared to 60 r.p.m. and account for the majority of the differences in internal power between the two frequencies (Ferguson *et al.* 2000). Clearly, the observation of almost a twofold difference in the level of internal power demonstrate that it essential to take into account the internal power when examining mechanical efficiency.

Muscle energy turnover at different contraction frequencies

It has been reported that energy turnover, as reflected by pulmonary $\dot{V}_{O_2}$, becomes higher as contraction frequency increases (Gaesser & Brooks, 1975; Coast & Welch, 1985; Sidossis *et al.* 1992; Chavarren & Calbet, 1999). It has, however, not been established whether the higher oxygen uptake observed in these studies was caused by more total work being performed at the higher contraction frequencies and whether oxygen uptake of the active muscles was higher. The present study showed that muscle oxygen uptake was elevated and the anaerobic energy turnover tended to be higher at the high contraction frequency when the same total power output was performed. Thus, the total metabolic rate was greater at the high contraction speed.

The total energy cost of a muscle contraction reflects the summed ATPase activity of muscle including myofibrillar ATPase as well as Ca^{2+} and membrane pump (ion) ATPases. It has been suggested that a significant part (20–50 %) of the total ATP utilised during a contraction may be used for ion transport related to muscle fibre activation and relaxation, independent of the ATP hydrolysis myofibrillar ATPase necessary for force generation between actin and myosin (Crow & Kushmerick, 1983). It is possible that both the number of contractions in a given time and the duration of each contraction may influence muscle energetics by altering the balance between energy required for activation processes in relation to contractile processes. The duration of force development for each knee extension phase was ∼0.5 s of the ∼1 s duty cycle at 60 r.p.m. corresponding to a contraction velocity of ∼180 deg s^{-1}. The duration of force development at 100 r.p.m. was ∼0.3 s for the ∼0.7 s duty cycle corresponding to a contraction velocity of ∼280 deg s^{-1}. Thus, with there being a higher number of contractions performed for a shorter duration the level of activation and relaxation is greater at 100 r.p.m., and consequently the ATP required for ion transport may be higher. Evidence for this is limited to isometric contraction. For example, Chastiotis *et al.* (1987) and Bergstrom & Hultman (1988) investigated electrically stimulated intermittent ischaemic isometric contractions of the human quadriceps muscle. They observed that energy turnover was greater during contractions of short compared to long duration when the total time of contraction was kept the same. In a fully perfused isolated canine gastrocnemius muscle preparation Hogan *et al.* (1998) demonstrated that the effective contraction time affected both aerobic and anaerobic energy turnover. Thus, muscle oxygen uptake, muscle lactate production and the rate of ATP utilisation were greater during short duration (0.25 s stimulation : 0.75 s rest) compared to long duration (1 s stimulation : 3 s rest) contractions.

The higher muscle energy turnover and oxygen uptake at the high contraction frequency may also have been due to different muscle fibre recruitment patterns or different efficiencies of fibres that were recruited during exercise at the two contractions frequencies. From *in vitro* studies, using either isolated muscles or singles muscle fibres, it has been observed that the efficiency of different fibre types is closely related to the velocity of contraction (di Prampero *et al.* 1988; Barclay, 1994, 1996; Reggiani *et al.* 1997; He *et al.* 2000). At low contraction velocities the efficiency of slow twitch (ST) fibres is higher than for fast twitch (FT) fibres and the reverse is observed at high speeds (He *et al.* 2000). In the present study the external power output at 60 r.p.m. was 41 W. This is about 15 % of the peak power output achieved during single-leg dynamic knee-extensor exercise (determined during the first seconds of maximal kicking) or about 60 % of the power output required to achieve the maximum $\dot{V}_{O_2}$ of the thigh (Bangsbo *et al.* 1990). It is probable that at 60 r.p.m. the majority of the required power could be generated by ST fibres with a small contribution from FT recruitment consequent upon an element of rate coding (Ivy *et al.* 1987). As previously discussed the duration of force development was much shorter at 100 compared to 60 r.p.m. It is therefore plausible that at 100 r.p.m. fewer ST fibres and more FT fibres were recruited, partly because the duty cycle at this contraction frequency may

have been too short for full activation of the ST fibres. A more pronounced activation of FT fibres may have caused the higher energy turnover and oxygen uptake at 100 r.p.m. Nevertheless, Gollnick *et al.* (1974) used histochemical glycogen depletion patterns as an indicator of fibre recruitment and were unable to detect any differences in fibre type recruitment patterns during cycle exercise at different contraction frequencies at various intensities. Similarly, Beelen *et al.* (1993) observed that both ST and FT fibres were recruited during cycle exercise (90 % $V_{O_2,max}$) at 60 and 120 r.p.m. Thus, more information is needed about the differential recruitment of muscle fibres at different contraction frequencies.

Another explanation for the larger energy turnover and muscle oxygen uptake at the high contraction frequency is that the muscle fibres that were recruited at the high contraction frequency were operating at a less than optimal velocity for maximal efficiency compared to the low contraction frequency. He *et al.* (2000) examined ATP consumption and efficiency of human single muscle fibres according to their different myosin isoform composition. The maximum efficiency was similar for all fibre types and was reached at a higher speed of shortening for the faster fibres. Thus, in the present study it may be that at the high contraction velocity the ST fibres that were active were working on the descending arm of their efficiency–velocity relationship and were consequently less efficient than at the lower contraction frequency.

Thigh blood flow and oxygen delivery at different contraction frequencies

Throughout the exercise period thigh blood flow was higher at the fast compared to the slow contraction frequency. In agreement with this finding, Sheriff *et al.* (1993) observed that in a canine hind-limb model doubling the contraction frequency during exercise resulted in a doubling in the initial rise in vascular conductance. One explanation for the elevated blood flow during the initial phase of exercise at 100 r.p.m. could be a greater contribution from the muscle pump in which rhythmic contractions cause a pumping action on the venous circulation in the skeletal muscle vascular bed (Laughlin, 1987). It is not fully understood how muscle perfusion is controlled to match the metabolic demands of the contracting muscle (Laughlin *et al.* 1996; Bangsbo & Hellsten, 1998; Rådegran & Hellsten, 2000; Saltin *et al.* 2000). Nevertheless, it is possible that the factors that may control skeletal muscle blood flow during exercise, such as potassium and adenosine (Bangsbo & Hellsten, 1998; Rådegrann & Hellsten, 2000), are more markedly changed at 100 r.p.m. in order to elevate blood flow to match the higher requirements for energy turnover at this frequency.

It is often discussed whether oxygen delivery limits muscle oxygen uptake in the initial phase of exercise (see Tschakovsky & Hughson, 1999). Interestingly, at 100 r.p.m. the difference between thigh O_2 delivery and O_2 uptake, which represents the O_2 not taken up, tended to be higher during the first 45 s compared to the remainder of the exercise (Fig. 6). A mismatch between O_2 delivery and uptake has also been observed within the first 15 s of intense knee-extensor exercise at 60 r.p.m. (Bangsbo *et al.* 2000). This was not evident at 60 r.p.m. in the present study probably due to the lack of measurements of O_2 extraction and blood flow during the very early phase of exercise. After 1 min and for the duration of the exercise O_2 not taken up at 100 r.p.m. was the same as at 60 r.p.m. (Fig. 6). Thus, these findings suggest that it took less than 15 s at 60 r.p.m. and some 30–45 s at 100 r.p.m. to adjust the effect of the muscle pump on blood flow to meet the metabolic requirements. Furthermore, it appears that oxygen delivery is in excess of oxygen uptake and does not limit oxygen utilisation at the onset of dynamic exercise.

The slow component of muscle oxygen uptake

During exercise at both contraction frequencies there was a gradual increase in thigh $\dot{V}_{O_2}$ from 3 min to the end of exercise. This has been termed the 'slow component' of $\dot{V}_{O_2}$ (Whipp & Wasserman, 1972). There was no difference in the magnitude of the $\dot{V}_{O_2}$ slow component between the two contraction frequencies. In agreement with these findings Barstow *et al.* (1996) measured pulmonary $\dot{V}_{O_2}$ during intense cycle exercise and observed that an increase in pedal frequency from 45 to 90 r.p.m. did not affect the magnitude of the slow component.

Several factors have been suggested to cause the slow oxygen component, including elevated temperature and increased muscle activity as well as altered motor unit and fibre type recruitment (see Gaesser & Poole, 1996). It has been speculated that increased muscle temperature may elevate oxygen uptake, which is possibly related to mitochondrial function (Brooks *et al.* 1971). For example, Willis & Jackman (1994) reported that with an increase in temperature of 3 °C the economy of oxidative phosphorylation in isolated rat and rabbit mitochondria was reduced by about 10 %. Muscle temperature, however, does not appear to be an important factor responsible for the slow component in the present study since muscle temperature was higher at 100 than at 60 r.p.m. without causing a higher slow component. This is in agreement with findings by Koga *et al.* (1997) who observed that during cycle exercise elevated muscle temperatures did not increase the slow component of pulmonary $\dot{V}_{O_2}$. It has also been suggested that the slow component could be a function of lactate accumulation and the associated acidosis (Whipp & Wasserman, 1986; Roston *et al.* 1987; Wasserman *et al.* 1991). However, in the present study no relationships were found between muscle lactate or venous blood pH and the change in muscle $\dot{V}_{O_2}$ over the last 7 min of exercise (Fig. 7). This suggests that a progressive increase in muscle lactate and lowering in muscle pH are not major determinants of the $\dot{V}_{O_2}$ slow component. This supports the mechanistic study

of Poole *et al.* (1994) in which infusion of lactate into the arterial blood supply of an electrically stimulated isolated canine gastrocnemius preparation did not increase oxygen uptake. It may be that a gradually altered recruitment pattern caused the slow rise in muscle $\dot{V}_{O_2}$, but whether a shift in fibre type recruitment occurs at the power output used in the present study is unclear. Further studies are needed to examine the origin of the slow oxygen component in humans.

Summary

It has been observed that oxygen uptake and rate of energy turnover during dynamic contractions in an isolated muscle group was greater at a contraction frequency of 100 compared to 60 r.p.m. Furthermore, muscle blood flow was higher at 100 r.p.m. and it took longer before oxygen delivery was adjusted to match the metabolic requirements at the high contraction frequency. Furthermore, oxygen uptake in the initial phase of exercise does not appear to be limited by oxygen delivery. Finally, it was observed that muscle oxygen uptake increased as exercise progressed in a manner that was not solely related to the increase in muscle temperature and lactate.

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