



Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise i humans

Quistorff, Bjørn; Krstrup, P.; González-Alonso, J.; Bangsbo, J.

Published in:
Journal of Physiology

Publication date:
2001

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Quistorff, B., Krstrup, P., González-Alonso, J., & Bangsbo, J. (2001). Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise i humans. *Journal of Physiology*, 536(3), 947-956.

Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise in humans

Peter Krstrup, José González-Alonso, Bjørn Quistorff and Jens Bangsbo

Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, Copenhagen, Denmark

(Received 9 March 2001; accepted after revision 27 June 2001)

1. The aim of the present study was to examine muscle heat production, oxygen uptake and anaerobic energy turnover throughout repeated intense exercise to test the hypotheses that (i) energy turnover is reduced when intense exercise is repeated and (ii) anaerobic energy production is diminished throughout repeated intense exercise.
2. Five subjects performed three 3 min intense one-legged knee-extensor exercise bouts (EX1, EX2 and EX3) at a power output of 65 ± 5 W (mean \pm S.E.M.), separated by 6 min rest periods. Muscle, femoral arterial and venous temperatures were measured continuously during exercise for the determination of muscle heat production. In addition, thigh blood flow was measured and femoral arterial and venous blood were sampled frequently during exercise for the determination of muscle oxygen uptake. Anaerobic energy turnover was estimated as the difference between total energy turnover and aerobic energy turnover.
3. Prior to exercise, the temperature of the quadriceps muscle was passively elevated to 37.02 ± 0.12 °C and it increased 0.97 ± 0.08 °C during EX1, which was higher ($P < 0.05$) than during EX2 (0.79 ± 0.05 °C) and EX3 (0.77 ± 0.06 °C). In EX1 the rate of muscle heat accumulation was higher ($P < 0.05$) during the first 120 s compared to EX2 and EX3, whereas the rate of heat release to the blood was greater ($P < 0.05$) throughout EX2 and EX3 compared to EX1. The rate of heat production, determined as the sum of heat accumulation and release, was the same in EX1, EX2 and EX3, and it increased ($P < 0.05$) from 86 ± 8 during the first 15 s to 157 ± 7 J s⁻¹ during the last 15 s of EX1.
4. Oxygen extraction was higher during the first 60 s of EX2 and EX3 than in EX1 and thigh oxygen uptake was elevated ($P < 0.05$) during the first 120 s of EX2 and throughout EX3 compared to EX1. The anaerobic energy production during the first 105 s of EX2 and 150 s of EX3 was lower ($P < 0.05$) than in EX1.
5. The present study demonstrates that when intense exercise is repeated muscle heat production is not changed, but muscle aerobic energy turnover is elevated and anaerobic energy production is reduced during the first minutes of exercise.

The effect of previous exercise on pulmonary oxygen uptake during intense exercise has been studied extensively (Gerbino *et al.* 1996; MacDonald *et al.* 1997). Less information exists about energy turnover in contracting human muscle during repeated intense exercise, where not only muscle oxygen uptake has to be measured, but also the anaerobic energy liberation of the contracting muscles. Various studies have included muscle metabolic measurements during repeated maximal cycle exercise and it has been suggested that the anaerobic energy production and energy turnover per work unit is lowered when intense exercise is repeated (Spriet *et al.* 1989; Gaitanos *et al.* 1993; Putman *et al.* 1995; Parolin *et al.* 1999). However, in these studies the aerobic energy production of the exercising muscles was not determined.

In addition, the work produced decreased when the exercise was repeated, which makes it difficult to relate the metabolic changes to the development of force. In studies using repeated knee-extensor exercise at a constant work rate, it has been observed that anaerobic energy production per work unit was reduced without a change in leg oxygen uptake (\dot{V}_{O_2}), when a second exercise bout was performed after 2.5 min and 60 min rest periods (Bangsbo *et al.* 1992*a,b*). However, in these studies the total energy turnover and anaerobic energy production in the various phases of exercise were not determined.

Another approach to determine total energy turnover is to measure heat production and power output. This method has been successfully implemented in *in vitro* studies of muscle fibres (Fenn, 1923; Hill & Woledge,

1962; Kushmerick *et al.* 1969) and *in vivo* examinations of exercising animals (Ardevol *et al.* 1998) and humans performing isometric contractions (Edwards *et al.* 1972, 1975; Saugen & Vøllestad, 1995). Recently, the method was utilized for quantification of energy fluxes in dynamically contracting muscle in man (González-Alonso *et al.* 2000). Total heat production during knee-extensor exercise was determined as heat stored in the contracting muscles and heat dissipated from the muscle. A major advantage with this approach is that heat production can be determined with a high time resolution without interfering with exercise. Furthermore, continuous determinations of the total heat production, power output and leg oxygen uptake during repeated knee-extensor exercise enables calculation of the oxygen deficit, representing the difference between the rate of energy turnover and oxygen uptake. Thereby, it is possible to compare the rate of anaerobic energy turnover in every phase of repeated intense exercise, and allow an examination of the effect of increased muscle temperature and lowered muscle pH, which have been suggested to increase ATP and energy turnover (Edwards *et al.* 1972; Curtin *et al.* 1988; Barclay *et al.* 1993; Willis & Jackman, 1994).

Thus, the aim of the present study was to examine muscle heat production, oxygen uptake and anaerobic energy turnover throughout repeated intense exercise to test the hypothesis that energy turnover is reduced when intense exercise is repeated and that anaerobic energy production is diminished in every phase of repeated intense exercise. Subjects performed three 3 min intense knee-extensor exercise bouts separated by 6 min rest periods. Muscle temperatures as well as femoral arterial and venous temperatures were measured continuously during the exercise periods. In addition, thigh blood flow was measured and arterial and femoral venous blood samples were collected frequently during the exercise bouts for determination of thigh oxygen uptake and lactate release.

METHODS

Subjects

Five, healthy, male subjects ranging in age from 22 to 25 years, with a mean (range) height of 180 (169–192) cm and body mass of 76.6 (55.4–91.8) kg participated in the experiment. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written informed consent to participate. The study conforms to the code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

Procedures and protocol

In the experiment subjects performed one-legged knee-extensor exercise in the supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscle (Andersen *et al.* 1985). Before the experiment the subjects practised the exercise on more than three separate occasions. On the morning of the experiment, subjects arrived after a light breakfast. With the subject

in the supine position, three catheters were placed with the Seldinger technique at the level of the inguinal ligament under local anaesthesia. In the resting leg, a catheter was placed in the femoral artery for blood sampling. In the exercising leg, catheters were placed in both the femoral artery and vein. Thermistors (Edslab probe 94–0.30–2.5F) were placed at the tip of these catheters for continuous measurements of arterial and venous blood temperature (sampling frequency of 100 Hz; response time < 0.8 s). The femoral venous catheter in the exercising leg was also used for blood sampling. In addition, seven thermistors (Edslab probe 94–0.30–2.5F) were inserted into the thigh muscles, as previously described (González-Alonso *et al.* 2000), at inclinations of 30, 45 and 60 deg related to muscle depth of 1.5–3.0 cm. Three thermistors were inserted in m. vastus lateralis (proximal, medial and distal portions), two in m. rectus femoris (medial and distal portions) and one in the distal portion of m. vastus medialis. The mean change in temperature of these quadriceps muscle portions was assumed to represent the change in m. vastus intermedius, since this muscle, located underneath m. rectus femoris, has been shown to have a similar temperature increase during such an exercise protocol (González-Alonso *et al.* 2000). In addition, one thermistor was placed in the medial portion of m. biceps femoris. Next to each of the seven muscle thermistor probes, a skin thermistor (MHC-40050-A, Ellab A/S, Denmark) was placed for monitoring of skin temperatures in 15 s intervals.

To minimize the heat loss from the exercising leg to the surrounding environment, thigh muscle, skin and blood temperatures were equalized to the core temperature ($\sim 37^\circ\text{C}$) by a water perfused thigh cuff, which was connected to a 70 l thermostat-regulated water-bath. The cuff was perfused with water at 41°C for 45–60 min until the muscle temperature was increased from resting temperatures of $34\text{--}35$ to $\sim 37^\circ\text{C}$. Then, the temperature of the perfusing water was reduced to 37°C for the rest of the experiment, which maintained the skin temperature at a level of $36.9\text{--}37.1^\circ\text{C}$. After an equilibrium period (> 10 min) the subject performed three 3 min knee-extensor exercise bouts (EX1, EX2 and EX3) with the experimental leg at external power outputs of 65.0 ± 4.8 (mean \pm S.E.M.), 66.1 ± 5.0 and 65.5 ± 4.8 W, respectively (average kicking frequency: 62 ± 1 , 63 ± 1 and 62 ± 1 r.p.m., respectively) each separated by a 6 min rest period. The intensity of the exercise was chosen so that the subjects would have been exhausted after about 4 min. Power output was continuously recorded during each of the exercise bouts. Before each of the exercise bouts the leg was passively moved for 30 s to accelerate the flywheel in order to obtain a constant power output from the onset of exercise. After a 60 min rest period, in which the thigh was kept heated, the entire exercise protocol was repeated with the same leg to allow for measurements of thigh blood flow (EX4, EX5 and EX6; see below). During each of the 3 min exercise periods a cuff just below the knee was inflated to 240 mmHg to avoid a contribution of blood from the lower leg. After each experiment all thermistors were immediately calibrated within the temperature range $35\text{--}40^\circ\text{C}$ against a mercury thermometer having a precision of 0.01°C .

Measurements

Blood samples were withdrawn from the femoral artery and vein at rest, during passive exercise and at approximately 15, 30, 50, 75, 125 and 175 s of EX1, EX2 and EX3. All blood samples were collected in 2 ml syringes and immediately placed in ice-cold water until analysed. Femoral venous blood flow was measured at rest, immediately prior to exercise and at the time intervals 0–10, 40–55, 80–95, 120–135 and 170–180 s of EX4, EX5 and EX6 by the thermodilution technique originally described by Andersen & Saltin (1985) and modified by González-Alonso *et al.* (2000). Briefly, ice-cold saline was infused at a constant rate of 120 ml min^{-1} for 15 s to

achieve a change in femoral venous blood temperature of 0.8–1.5°C after an initial stabilization period of 5 s. Thigh blood flow was not measured during the first three exercise bouts in order to avoid the effect of infusing cold saline on venous and arterial blood temperatures. Previous studies from this laboratory indicate that the kinetics and magnitude of femoral venous blood flow is the same when intense knee-extensor exercise is repeated after 1 h of recovery (Bangsbo *et al.* 1992b, 2001).

Blood analysis. Oxygen saturation of blood and haemoglobin concentration were determined spectrophotometrically (OSM-3 Hemoximeter, Radiometer, Copenhagen, Denmark). The Hemoximeter was calibrated spectrophotometrically by the cyanomethaemoglobin method (Drabkin & Austin, 1935). Blood pH was measured with the Astrup technique (ABL 30, Radiometer, Copenhagen). A part of the blood sample (100 μ l) was haemolysed within 10 s of sampling using a 1:1 dilution with a buffer solution (Yellow Spring Instruments, Yellow Springs, OH, USA) to which was added 20 g l⁻¹ Triton X-100 for analysis of lactate using a lactate analyser (model 23, Yellow Spring Instruments; Foxdal *et al.* 1992).

Muscle mass. Muscle mass was determined by measurements of the muscle volume by magnetic resonance imaging (MRI) performed on a Siemens 1.5 T MAGNETOM vision scanner (Siemens, Germany) as described earlier (Rasband & Bright, 1995; González-Alonso *et al.* 2000). The volume values were converted into muscle mass using a specific density of muscle of 1.043 g cm⁻³ (von Döbeln, 1956). The mean mass of the knee-extensor muscles, including the tensor fascia latae, was 2.68 kg, with a range of 2.14–3.27 kg, while the mean mass of inactive muscles in the thigh (hamstrings and adductors) was 2.97 kg, with a range of 2.48–3.61 kg.

Calculations

Heat accumulation in active muscles. The rate of heat accumulation in the active muscles (i.e. quadriceps and tensor fascia latae) was calculated for 15 s intervals by multiplying the mean increase in temperature (°C) of all muscle portions in 15 s by the muscle mass (kg) and the specific heat of the muscle at 37.5°C (3.59 kJ kg⁻¹ °C⁻¹), assuming an average solid content of 23%. The same principle was used to determine the rate of heat storage in inactive muscles. The active muscle mass was corrected by the estimated increases in muscle volume due to fluid accumulation observed during intense knee-extensor exercise (Bangsbo *et al.* 1992a), representing an increase of 0.26 \pm 0.04 kg.

Heat release to the blood. The rate of heat release to the blood was calculated each second by multiplying the venous–arterial temperature difference (°C) by thigh blood flow (l s⁻¹) and the specific heat of the blood at 37.5°C (3.61 kJ l⁻¹ °C⁻¹; haematocrit ~45%). The thigh blood flow was estimated each second from an individual blood flow curve constructed by linear connection of the consecutive measured blood flow values in each exercise bout. Thereafter, the average rate of heat release during each 15 s interval was calculated.

Heat production. The rate of heat production was calculated as the sum of the rate of heat accumulation in the active muscles, the rate of heat removal by the blood and an additional heat production (see below). The additional rate of heat production was estimated as heat loss to the surrounding skin by the processes of conductance and convection as well as heat transfer from the thigh to the body core by the lymph drainage as described earlier (González-Alonso *et al.* 2000). The estimated heat loss during the first 15 s of EX1, EX2 and EX3 was 3, 7 and 9 J s⁻¹, respectively, and it increased to 13, 15 and 16 J s⁻¹ during the last 15 s of EX1, EX2 and EX3, respectively.

Energy turnover and mechanical efficiency. The rate of energy turnover was estimated by adding the rate of heat production and mechanical power output (W). Mechanical power output was the sum

of external work and internal work, which has been estimated to be 17 W and constant throughout exercise (Ferguson *et al.* 2000). Mechanical efficiency was calculated by dividing the mechanical power output by the total energy turnover.

Thigh oxygen uptake and lactate release. Thigh oxygen uptake (\dot{V}_{O_2}) and net lactate release were calculated by multiplying the blood flow by the difference between the femoral arterial and venous concentrations. Oxygen uptake was converted into kilojoules by multiplying by 21.2 kJ l⁻¹ O₂, assuming that muscle glycogen was the only substrate during the exercise (Bangsbo *et al.* 1992b).

Anaerobic energy turnover. Anaerobic energy turnover was estimated in 15 s intervals as the oxygen deficit, i.e. the difference between net aerobic energy production and net energy production.

Mean transit time correction. To obtain measurements of oxygen uptake and lactate release at the capillary level and relate changes in blood temperatures to the time of accumulation of heat in the muscle, corrections were made for the blood transit time from the capillaries to the collection points in the femoral artery and vein. This correction has significant importance in the initial phase of exercise, where blood flow, oxygen extraction and blood temperatures all rise progressively. Arterio-venous mean transit times of 11, 7, 6 and 6 s after 5, 25, 65, 152 s were used for all exercise bouts, with 1/3 of the time representing the time from artery to capillary (Bangsbo *et al.* 2000).

Statistics

Two way analysis of variance (ANOVA) with repeated measures was used for evaluation of changes during the exercises as well as between EX1, EX2 and EX3. If a significant *F* value was observed then the Newman–Keuls *post hoc* tests were used to locate the differences. A significance level of 0.05 was chosen. The standard error of the mean (\pm S.E.M.) is only given in the text where this value cannot be obtained from a figure.

RESULTS

Muscle heat accumulation

The temperature in the quadriceps muscles increased from 37.02 to 37.99°C during EX1 (Fig. 1A). The quadriceps temperature before EX2 and EX3 was around 37.5°C and it increased to approximately 38.2°C, with the temperatures being higher ($P < 0.05$) than in EX1. The rise in quadriceps temperature of 0.97 \pm 0.08°C during EX1 was higher ($P < 0.05$) than during EX2 and EX3 (0.79 \pm 0.05 and 0.77 \pm 0.06°C, respectively). Thus, in EX1 the rate of muscle heat accumulation was higher ($P < 0.05$) during the first 120 s and the last 30 s of exercise compared to EX2 and EX3 (Fig. 1B).

Thigh blood flow

The passive exercise before EX1 elevated the thigh blood flow from 0.43 to 1.96 l min⁻¹ and it increased further ($P < 0.05$) to 3.11 l min⁻¹ after 42 s and 4.32 l min⁻¹ at the end of exercise (Fig. 2). Before and during the first 90 s of EX2 and during EX3, thigh blood flow was higher ($P < 0.05$) than in EX1 (Fig. 2).

Muscle heat release to the blood

At the start of EX1, femoral arterial and venous temperatures were 36.89 and 36.86°C, respectively, and during EX1 they increased to 37.16 and 37.50°C,

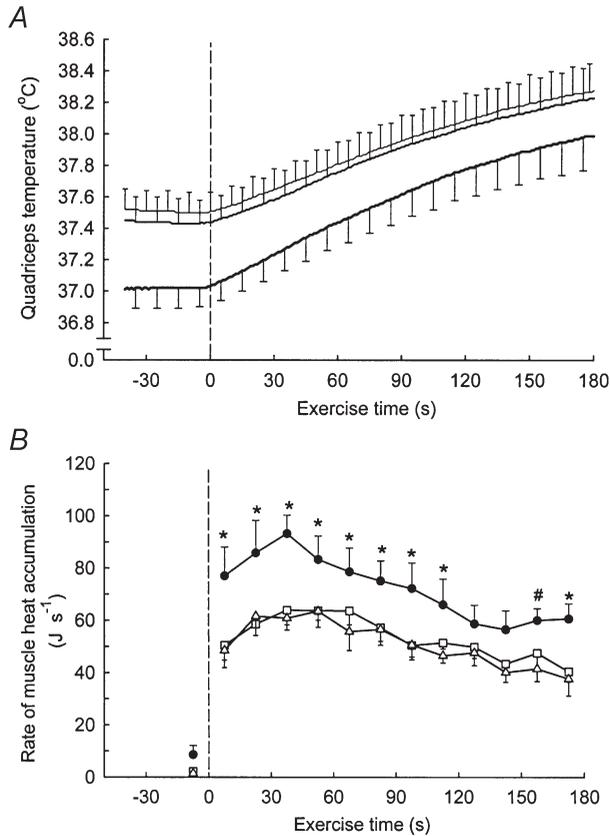


Figure 1. Quadriceps temperature (A) and rate of heat accumulation in muscle (B) before and during repeated 3 min bouts of intense knee-extensor exercise

Means \pm S.E.M. are given. * EX1 significantly ($P < 0.05$) different from EX2 and EX3; # EX1 significantly ($P < 0.05$) different from EX3. A, EX1: thick line; EX2: medium-weight line; EX3: thin line. B, EX1: ●; EX2: □; EX3: △.

respectively. During EX2 and EX3 the blood temperatures were higher ($P < 0.05$) than during EX1, and the venous–arterial temperature difference tended to be greater during EX2 and EX3 than in EX1, being, respectively, 0.40 ± 0.05 and 0.41 ± 0.05 °C compared to 0.34 ± 0.03 °C at the end of exercise (Fig. 3). The rate of heat release to the blood increased progressively during each exercise bout and was higher ($P < 0.05$) throughout EX2 and EX3 compared to EX1 (Fig. 4).

Muscle heat production

The rate of heat production, calculated as the sum of heat accumulation, heat release and additional heat production (see Methods), was 86 J s^{-1} during the first 15 s of EX1, and increased ($P < 0.05$) progressively to 121 J s^{-1} after 30–45 s and 157 J s^{-1} during the last 15 s of EX1 (Fig. 5A). During EX2 and EX3 the magnitude and the

changes in heat production were similar to those for EX1 (Fig. 5A). The total heat production was 22.8 ± 1.1 , 23.8 ± 1.7 and 25.9 ± 2.6 kJ in EX1, EX2 and EX3, respectively.

Energy turnover and mechanical efficiency

The rate of energy turnover followed the pattern of changes in heat production, since the power output was essentially constant in all the three exercise bouts. Thus, in EX1 the rate of energy production increased ($P < 0.05$) from 172 ± 8 during the first 15 s to $237 \pm 7 \text{ J s}^{-1}$ during the last 15 s of EX1. This was similar to the changes in EX2 (162 ± 4 to $248 \pm 9 \text{ J s}^{-1}$) and EX3 (166 ± 8 to $264 \pm 23 \text{ J s}^{-1}$), even though energy production tended ($P = 0.1–0.2$) to be higher towards the end of EX3 compared to EX1. The total energy production in EX1, EX2 and EX3 was 37.6 ± 1.4 , 38.7 ± 1.9 and 40.7 ± 2.9 kJ,

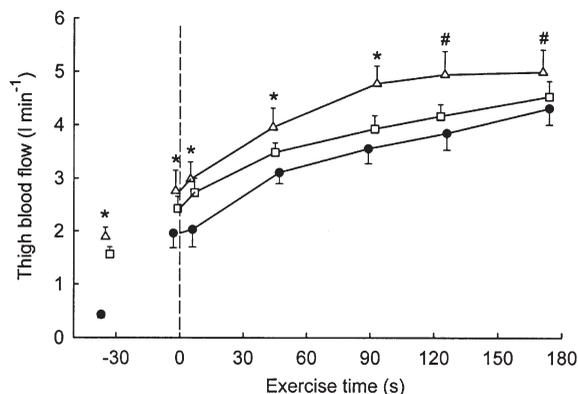
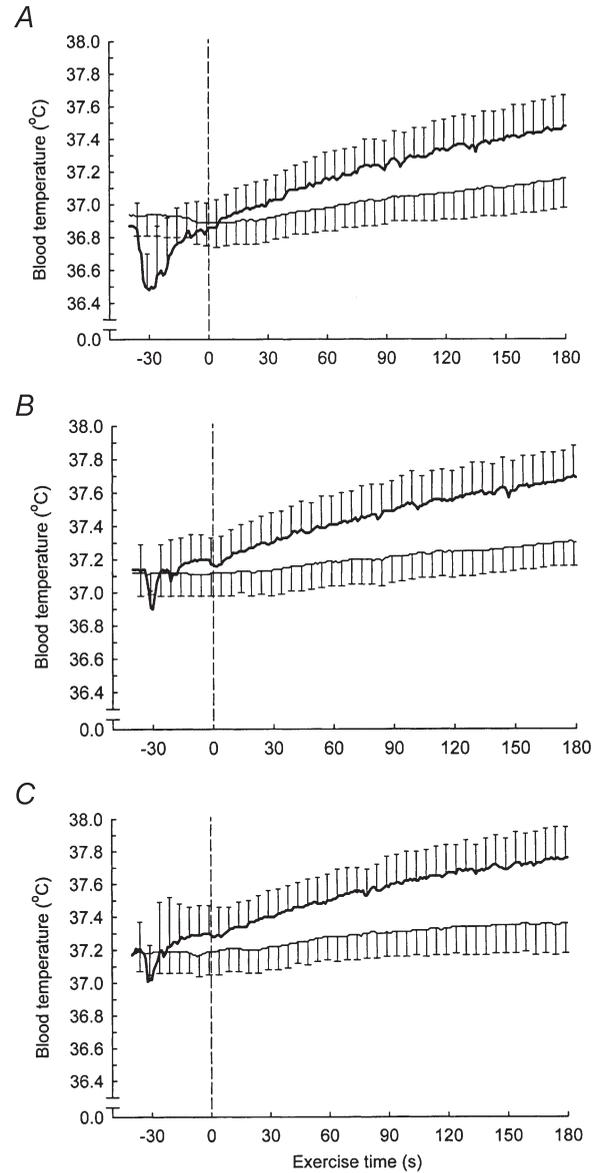


Figure 2. Thigh blood flow before and during repeated 3 min bouts of intense knee-extensor exercise

Means \pm S.E.M. are given. * EX1 significantly ($P < 0.05$) different from EX2 and EX3. # EX1 significantly ($P < 0.05$) different from EX3. EX1: ●; EX2: □; EX3: △.

Figure 3. Blood temperatures before and during repeated 3 min bouts of intense knee-extensor exercise

Arterial (thin line) and venous (thick line) temperatures in EX1 (A), EX2 (B) and EX3 (C). Means \pm S.E.M. are given.



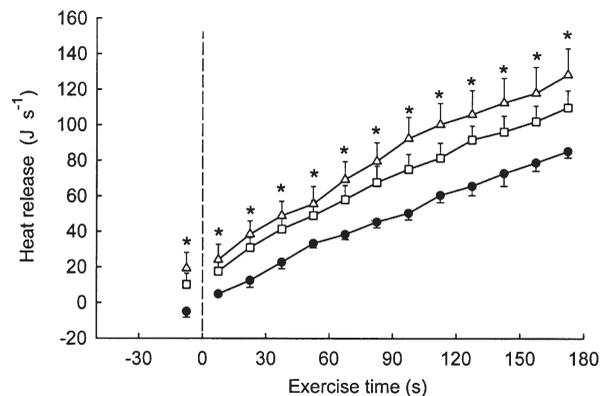
respectively. The estimated mechanical efficiency in EX1, EX2 and EX3 declined ($P < 0.05$) from around 51 % during the first 15 s of exercise to 41 % after 30–45 s and further ($P < 0.05$) to 33 % during the last 15 s of exercise (Fig. 5B), with mean values for EX1, EX2 and EX3 of 39.3 ± 2.0 , 38.8 ± 2.5 and $37.0 \pm 2.5\%$, respectively.

Aerobic energy turnover

The arterial–venous oxygen difference increased ($P < 0.05$) from 23 ml l^{-1} immediately before to 153 ml l^{-1} at the end of EX1 (Fig. 6A). In EX2 and EX3 the arterial–venous oxygen difference was higher ($P < 0.05$) during the first 43 s of exercise compared to EX1 (Fig. 6A). During EX1

Figure 4. The rate of heat release to the blood before and during repeated 3 min bouts of intense knee-extensor exercise

Means \pm S.E.M. are given. * EX1 significantly ($P < 0.05$) different from EX2 and EX3. EX1: ●; EX2: □; EX3: △.



thigh oxygen uptake increased ($P < 0.05$) from 0.05 l min^{-1} before exercise to 0.40 l min^{-1} after 43 s, reaching 0.66 l min^{-1} at the end of exercise (Fig. 6B). During the first 120 s of EX2 and during EX3 thigh oxygen uptake was higher ($P < 0.05$) than during EX1 (Fig. 6B).

Anaerobic energy turnover

Anaerobic energy turnover, estimated as the difference between total net energy turnover and net aerobic energy turnover, was 123 J s^{-1} during the first 15 s of EX1 and decreased ($P < 0.05$) progressively to 62 J s^{-1} after 45–60 s and 19 J s^{-1} during the last 15 s of EX1 (Fig. 7). During the first 105 s of EX2 and 150 s of EX3 anaerobic energy production was lower ($P < 0.05$) compared to EX1 (Fig. 7). Thus, total anaerobic energy turnover was lower ($P < 0.05$) in EX2 and EX3 compared to EX1, being, respectively, 4.9 ± 1.7 and $0.7 \pm 2.9 \text{ kJ}$ vs. $8.7 \pm 1.9 \text{ kJ}$ corresponding

to 14 ± 5 and $2 \pm 7\%$ vs. $23 \pm 5\%$ of total energy turnover.

No net lactate release from the thigh was observed before EX1, but it increased ($P < 0.05$) to $3.9 \pm 0.6 \text{ mmol min}^{-1}$ after 43 s and further to $11.8 \pm 1.1 \text{ mmol min}^{-1}$ at the end of EX1. Before and during the first 23 s of EX2 and EX3, net lactate release was higher ($P < 0.05$) than during EX1, after which no differences were observed.

Blood pH

Arterial pH decreased ($P < 0.05$) from 7.41 ± 0.02 immediately before to 7.37 ± 0.02 at the end of EX1. Femoral venous pH increased ($P < 0.05$) from 7.37 ± 0.02 immediately prior to exercise to 7.41 ± 0.02 after 8 s, after which it decreased ($P < 0.05$) to 7.35 ± 0.01 after 23 s and 7.14 ± 0.01 at the end of EX1. Before and during the first 23 s of EX2 and EX3 arterial and femoral venous pH was lower ($P < 0.05$) than during EX1, whereas femoral venous pH became higher ($P < 0.05$) during the last 120 s of EX3 compared to EX1, reaching 7.17 ± 0.01 at the end of EX3.

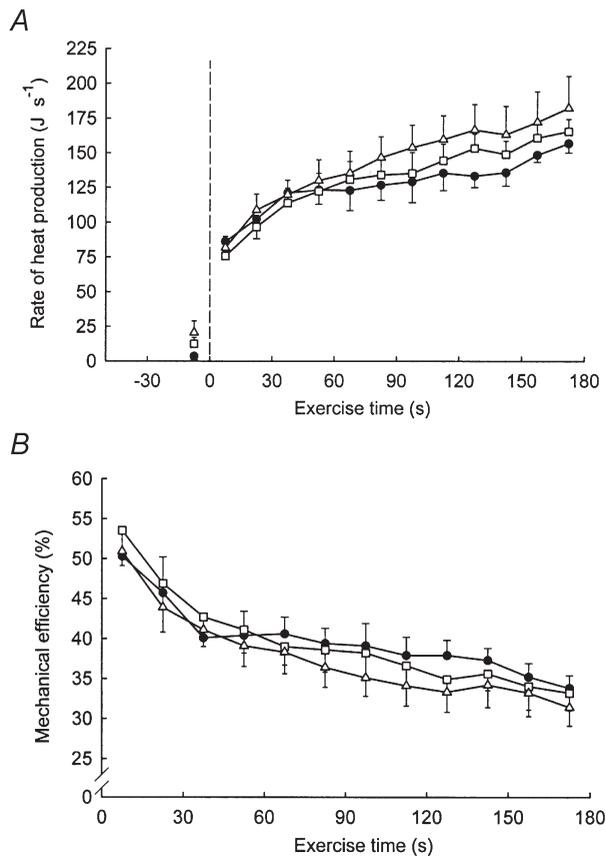


Figure 5. Rate of muscle heat production (*A*) and muscle mechanical efficiency (*B*) before and during repeated 3 min bouts of intense knee-extensor exercise

A, heat production calculated as the sum of rate of heat accumulation in the muscle and heat release to the blood. *B*, muscle mechanical efficiency determined as total work divided by total energy production. Means \pm S.E.M. are given. EX1: ●; EX2: □; EX3: △.

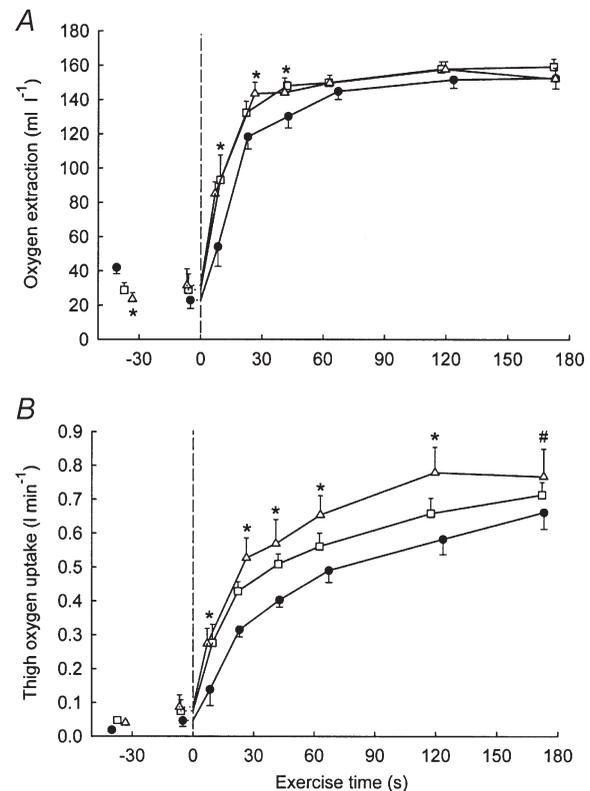


Figure 6. Thigh oxygen extraction (*A*) and thigh oxygen uptake (*B*) during repeated 3 min bouts of intense knee-extensor exercise

Means \pm S.E.M. are given. * EX1 significantly ($P < 0.05$) different from EX2 and EX3. # EX1 significantly ($P < 0.05$) different from EX3. EX1: ●; EX2: □; EX3: △.

DISCUSSION

The results of the present study did not confirm the hypothesis that energy turnover is reduced when intense exercise is repeated, since muscle heat production was the same and increased in a similar way in the three exercise bouts performed at the same work intensity. Thus, mechanical efficiency, expressed as work per energy turnover, was similar in the first, second and third bouts of intense exercise, but did appear to decline within each exercise bout. It was also demonstrated that anaerobic energy production was reduced throughout repeated exercise in association with a higher aerobic energy contribution.

Heat production during repeated intense exercise

Muscle heat production increased in a similar way during each of the three exercise bouts, since a lower muscle heat accumulation in EX2 and EX3 compared to EX1 was compensated by a higher heat release to the blood. The lower heat release in EX1 than in EX2 and EX3 was probably due to a $\sim 0.5^{\circ}\text{C}$ lower muscle temperature in EX1, resulting in a lower muscle-to-blood temperature gradient. Thus, the present findings do not support the hypothesis that energy turnover per work unit is lowered when intense exercise is repeated. It has been observed that the mean rate of energy turnover was lower when intense knee-extensor exercise was repeated after 2.5 min and 1 h of recovery (Bangsbo *et al.* 1992*a,b*). These observations can be explained by the longer exercise duration of the first bout compared to the second, since the present study showed that mechanical efficiency decreases as exercise progresses.

The increasing heat production during intense exercise could be due to a progressive increase in ATP turnover, since it has been estimated from muscle metabolic measurements and oxygen uptake that ATP turnover is lower in the first 15 s of intense exercise than in the remaining part of a 3 min exercise bout (Bangsbo *et al.* 2001). Based on *in vitro* studies it has been proposed that factors such as pH and elevated temperature cause a higher energy turnover as exercise progresses (Cooke *et al.* 1988; Curtin *et al.* 1988; Barclay *et al.* 1993; Willis & Jackman, 1994; Wolosker *et al.* 1997). In the present study, muscle temperature increased by about 1°C during the exercise bouts, which may have elevated the heat production as exercise continued, since it has also been observed that prior passive heating increases the energy cost of isometric contractions (Edwards *et al.* 1972) and oxygen uptake during submaximal cycle exercise (Ferguson *et al.* 1999). However, the muscle temperatures were about 0.5°C higher before and during EX2 and EX3 compared to EX1 without affecting the rate of heat production, suggesting that any effect of temperature is minor. It is likely that muscle pH was lowered prior to EX2 and EX3 compared to EX1 as reflected in the lower femoral venous pH at the start of exercise, which could

have elevated the heat and ATP production. However, the observation that heat production in the initial phase of repeated exercise was not altered suggests that pH does not have any significant effect on energy production. This suggestion is in agreement with the observation that mean ATP production during intense exercise is not affected by a lowered muscle pH, caused by preceding

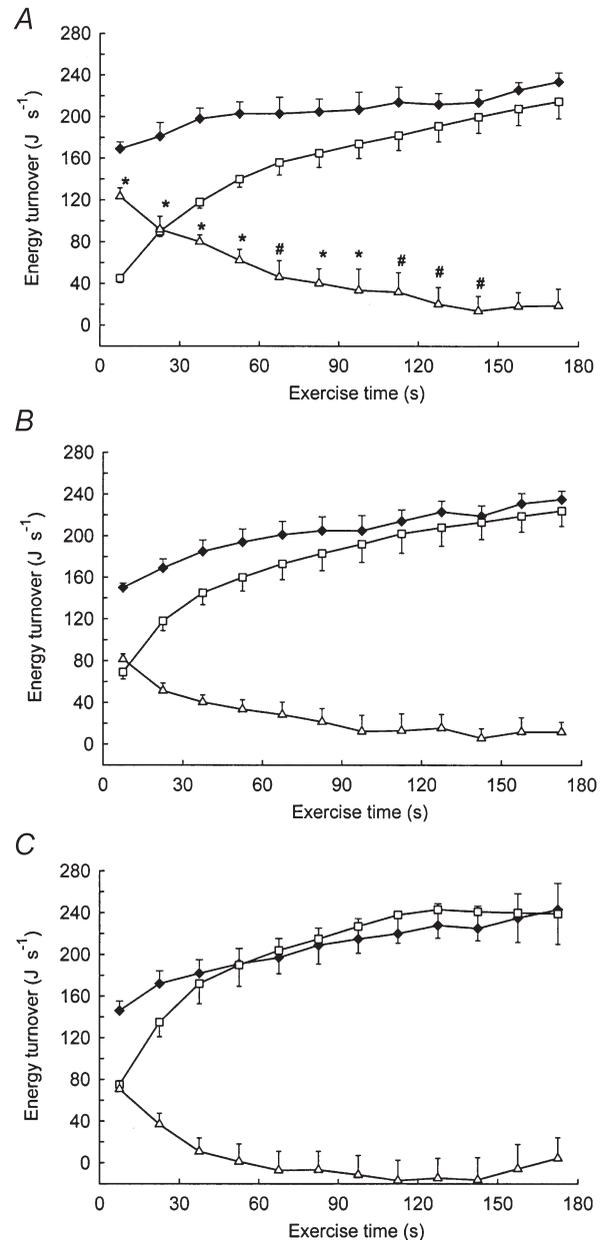


Figure 7. Anaerobic energy production (Δ) calculated as the difference between total net energy turnover (\blacklozenge ; sum of net heat production and power output) and net aerobic energy production (\square) in EX1 (A), EX2 (B) and EX3 (C)

Means \pm S.E.M. are given. * EX1 significantly ($P < 0.05$) different from EX2 and EX3. # EX1 significantly ($P < 0.05$) different from EX3.

intense arm exercise (Bangsbo *et al.* 1996). Thus, the present data suggest that neither an elevated muscle temperature nor a lowered muscle pH has any significant effect on muscle heat production.

An additional explanation of the increase in heat production as intense exercise continues could be that the rate of creatine phosphate (CP) utilization decreases and rate of oxidative phosphorylation increases as exercise progresses (Bangsbo *et al.* 2001), since it has been observed *in vitro* that heat liberation per mole of ATP produced through the net breakdown of CP was significantly lower than for glycolysis and oxidative phosphorylation (~55 *vs.* 67 and 95 kJ; Walsh & Woledge, 1970; Curtin & Woledge, 1978; Hinckle & Yu, 1979). This is not supported by the finding that the elevated oxygen uptake in the first 60 s of EX2 and EX3 was not reflected in a similar increase in heat production and energy turnover (Figs 5–7). It is possible, however, that the rate of thigh ATP production was reduced in the first phase of EX3 compared to EX1 (Bangsbo *et al.* 2001), thereby counteracting any effect of a lower efficiency of oxidative phosphorylation. In the last phase of exercise, the higher oxygen uptake in EX3 compared to EX1 was associated with a higher heat production. Thus, it is possible that differences in the efficiency of the metabolic pathways used during the intense exercise are in part causing the higher heat production as intense exercise progresses, but further studies are needed to address this question.

In the present study, the mechanical efficiency decreased from around 50% in the initial phase of exercise to 31–34% at the end of the three exercise bouts. The high initial efficiency is in agreement with findings in another study using intense knee-extensor exercise and determination of muscle metabolites (Bangsbo *et al.* 2001). This can be explained by a low ATP turnover at the onset of exercise and/or low heat liberation related to the anaerobic ATP resynthesis (as discussed above). In the last phase of exercise where oxidative phosphorylation provides most of the energy, the efficiency is of the same magnitude as during steady-state submaximal knee-extensor exercise (25–30%; Andersen & Saltin, 1985; Bangsbo *et al.* 1990) and bicycle exercise (30–34%; Luthanen *et al.* 1987; Poole *et al.* 1992), when the internal work is taken into account.

Anaerobic energy production during repeated exercise

It has previously been observed, based on metabolic measurements, that total muscle anaerobic energy turnover is reduced when intense exercise is repeated, but it has been unclear in what phases of exercise the reduction occurs (Spriet *et al.* 1989; Bangsbo *et al.* 1992*a,b*; Gaitanos *et al.* 1993; Putman *et al.* 1995). The present study used another approach, comparing total energy turnover and oxygen uptake, allowing a detailed analysis of the anaerobic energy production throughout intense exercise.

That the method has a high validity was confirmed by the finding that the values for the anaerobic energy turnover during the entire exercise periods corresponded well with what has previously been observed in studies using metabolic measurements. The anaerobic energy production for EX1 and EX2 was 23 and 14% of total energy turnover, respectively, values which are similar to those observed (22 *vs.* 15%) in another knee-extensor exercise study with a similar protocol (Bangsbo *et al.* 2001). The reduction in anaerobic energy turnover between EX1 and EX2 of 42% was also of a magnitude that is similar to studies using metabolic measurements (24–50%; Bangsbo *et al.* 1992*a,b*, 2001). The present study demonstrated further that the anaerobic energy contribution was lower in every phase until 150 s of repeated exercise (Fig. 7). The observed reduction in anaerobic energy production was most probably due to a lowering of glycolysis, since the rate of CP degradation has been shown to be similar when intense exercise is repeated (Bangsbo *et al.* 1992*a,b*, 2001). Using data for CP and ATP utilization obtained in similar studies (Hellsten *et al.* 1999; Bangsbo *et al.* 2001) it can be estimated that the rate of ATP production from glycolysis was 1.21 mmol ATP s⁻¹ during the first 15 s in EX1 compared to 0.58 and 0.42 mmol ATP s⁻¹ in EX2 and EX3, respectively, whereas it was 0.28 mmol ATP s⁻¹ during the last 15 s of exercise in EX1, 0.17 mmol ATP s⁻¹ in EX2 and negligible in EX3. The cause of the reduction in glycolysis when exercise is repeated has been discussed (Bangsbo *et al.* 1992*b*). Elevated H⁺ concentrations have been suggested to cause a lowering of the rate of glycolysis when intense exercise is repeated (Danforth, 1965; Chasiotis, 1983; Parolin *et al.* 1999). It is possible, as previously discussed, that muscle pH was lowered prior to EX2 and EX3 compared to EX1, but it is likely that the difference disappeared early in the exercise, since blood pH was the same in the three exercise bouts after about 25 s of exercise. Actually, muscle pH at the end of EX2 and EX3 may have been higher than at the end of EX1 (Bangsbo *et al.* 1992*a*) as reflected by the higher femoral venous pH at the end of EX3. Thus, the finding of a significant difference in anaerobic energy throughout exercise appears not to be explained by a difference in the level of acidity. The reduction in glycolysis when the intense exercise was repeated was closely related to the greater oxidation and it may be that muscle glycolysis is inhibited throughout EX2 and EX3 by a compound, such as free ADP, that is lowered due to the greater oxidative phosphorylation. Further studies are needed to investigate such a coupling.

Muscle oxygen uptake during repeated exercise

The present study showed that muscle oxygen extraction and oxygen uptake were elevated in the initial phase when exercise is repeated, with no difference between the second and third exercise bout. These findings suggest that there is a local limitation in muscle oxygen uptake in the initial phase of the first exercise bout, and the question is what causes such a restriction.

It has been suggested that the more rapid rise in oxygen uptake when exercise is repeated is due to a lowered muscle pH (Germino *et al.* 1996). Prior to the second and third exercise bout, muscle pH was probably lower than before the first exercise, which may have influenced muscle respiration. However, the difference did not last as long as the difference in oxygen extraction, indicating that muscle pH is not a major factor in accelerating the oxygen utilization after prior exercise. The difference is not likely to be caused by the higher temperature at the start of EX2 and EX3 compared to EX1, since Koga *et al.* (1997) did not observe any effect on pulmonary \dot{V}_{O_2} kinetics by elevating the muscle temperature. Thus, neither a lowered muscle pH nor an elevated muscle temperature seems to explain the higher oxygen extraction when exercise is repeated, and it still appears unclear what causes the low oxygen extraction in the initial phase of exercise (Bangsbo *et al.* 2000).

In EX1 thigh oxygen uptake increased progressively as exercise continued but not as rapidly as in EX2 and EX3. In addition, the level of oxygen uptake reached at the end of EX1 was significantly lower than at the end of EX3. These findings suggest that muscle oxygen uptake is also limited in the last 2 min of EX1. The progressive increase in oxygen uptake in the later phase (last 2 min) of EX1 was due to an increase in blood flow and the difference in oxygen uptake between EX1 and EX3 in this phase of exercise was also exclusively due to a higher blood flow in EX3 compared to EX1. Apparently, the elevated blood flow was supplying metabolically active muscle fibres since the arterial–venous O_2 difference did not change during the last phase of EX1 and reached the same level as at the end of EX2 and EX3. The higher muscle oxygen uptakes in EX2 and EX3 may have been caused by enhanced recruitment of motor units. Alternatively, the blood delivery to the active muscle fibres may have been higher. In this case, the observation of the same oxygen extraction in last phase of EX1 and EX3 shows that mitochondrial activity in each muscle cell can be elevated and that a limitation exists in the first exercise bout. In contradiction to this explanation is the finding that the oxygen delivery in both EX1 and EX3 considerably exceeded the oxygen uptake (reflected in the relative high femoral venous O_2 contents). It is also possible that the same number of fibres are recruited in the three exercise bouts and that some non-recruited, but previously contracted, muscle fibres had a markedly elevated oxygen uptake and energy turnover. The observation that muscle heat production and oxygen uptake prior to EX2 and EX3 were higher compared to EX1 shows that recovery processes are going on in the previously exercised muscle (Figs 5 and 6). The difference between energy turnover before EX3 and EX1 was 17 J s^{-1} , which is of the same magnitude as the difference observed at the end of EX3 and EX1. This could suggest that pre-exercise energy turnover continued during exercise independently of the energy production during exercise. It is, however,

unlikely that the recovery processes before EX2 and EX3 are continuing to the same extent during exercise. Nevertheless, the present study demonstrates that oxygen uptake for an isolated exercising muscle does not reach a peak level during a 3 min exercise period, suggesting that oxygen utilization is inhibited.

Summary

The present data show that muscle heat production and energy turnover are unaltered when intense exercise is repeated at the same work intensity but declines within each exercise bout. Furthermore, muscle oxygen uptake is elevated and anaerobic energy production is reduced in every phase of repeated intense exercise.

- ANDERSEN, P., ADAMS, R. P., SJØGAARD, G., THORBOE, A. & SALTIN, B. (1985). Dynamic knee extension as a model for the study of an isolated exercising muscle in man. *Journal of Applied Physiology* **59**, 1647–1653.
- ANDERSEN, P. & SALTIN, B. (1985). Maximal perfusion of skeletal muscle in man. *Journal of Physiology* **366**, 233–249.
- ARDEVOL, A., ADAN, C., REMESAR, X., FERNÁNDEZ-LÓPEZ, J. A. & ALEMANY, M. (1998). Hind leg heat balance in obese Zucker rats during exercise. *Pflügers Archiv* **435**, 454–464.
- BANGSBO, J., GOLLNICK, P. D., GRAHAM, T. E., JUEL, C., KIENS, B., MIZUNO, M. & SALTIN, B. (1990). Anaerobic energy production and O_2 deficit-debt relationship during exhaustive exercise in humans. *Journal of Physiology* **422**, 539–559.
- BANGSBO, J., GRAHAM T. E., JOHANSEN, L., STRANGE, S., CHRISTENSEN, C. & SALTIN, B. (1992a). Elevated muscle acidity and energy production during exhaustive exercise in man. *American Journal of Physiology* **263**, R881–899.
- BANGSBO, J., GRAHAM, T. E., KIENS, B. & SALTIN, B. (1992b). Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *Journal of Physiology* **451**, 205–227.
- BANGSBO, J., KRUSTRUP, P., GONZÁLEZ-ALONSO, J., BOUSHEL, R. & SALTIN, B. (2000). Muscle oxygen kinetics at onset of intense dynamic exercise in humans. *American Journal of Physiology* **279**, R899–906.
- BANGSBO, J., KRUSTRUP, P., GONZALEZ-ALONSO, J. & SALTIN, B. (2001). ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. *American Journal of Physiology* **280**, E956–964.
- BANGSBO, J., MADSEN, K., KIENS, B. & RICHTER, E. A. (1996). Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *Journal of Physiology* **495**, 587–596.
- BARCLAY, C. J., CONSTABLE, J. K. & GIBBS, C. L. (1993). Energetics of fast- and slow-twitch muscles of the mouse. *Journal of Physiology* **472**, 61–80.
- CHASIOTIS, D. (1983). The regulation of glycogen breakdown in human skeletal muscle. *Acta Physiologica Scandinavica (Supplementum)* **518**, 1–68.
- COOKE, R., FRANKS, K., LUCIANI, G. B. & PATE, E. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *Journal of Physiology* **395**, 77–97.
- CURTIN, N. A., KOMETANI, K. & WOLEDGE, R. C. (1988). Effect of intracellular pH on force and heat production in isometric contraction of frog muscle fibres. *Journal of Physiology* **396**, 93–104.

- CURTIN, N. A. & WOLEDGE, R. C. (1978). Energy changes and muscular contraction. *Physiological Reviews* **58**, 690–761.
- DANFORTH, W. H. (1965). Activation of glycolytic pathways in muscle. In *Control of Energy Metabolism*, ed. CHANCE, B., ESTRABROOK, R. W. & WILLIAMSON, J. R., pp. 287–297. Academic Press, New York.
- DRABKIN, D. L. & AUSTIN, J. H. (1935). Spectrophometric studies II. Preparations from washed blood cells, nitric oxide hemoglobin and sulhemoglobin. *Journal of Biological Chemistry* **122**, 51–56.
- EDWARDS, R. H. T., HARRIS, R. C., HULTMAN, E., KALJSER, L., KOH, D. & NORDESIÖ, L.-O. (1972). Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *Journal of Physiology* **220**, 335–352.
- EDWARDS, R. H. T., HILL, D. K. & JONES, D. A. (1975). Heat production and chemical changes during isometric contractions of the human quadriceps muscle. *Journal of Physiology* **251**, 303–315.
- FENN, W. O. (1923). A quantitative comparison between the energy liberated and the work performed by an isolated sartorius of the frog. *Journal of Physiology* **58**, 175–203.
- FERGUSON, R. A., AAGAARD, P., BALL, D., SARGEANT, A. J. & BANGSBO, J. (2000). Total power output generated during dynamic knee extensor exercise at different contraction frequencies. *Journal of Applied Physiology* **89**, 1912–1918.
- FERGUSON, R. A., BALL, D. & SARGEANT, A. J. (1999). Effect of muscle temperature on oxygen consumption and metabolic responses to whole body exercise in humans at different movement frequencies. *Journal of Physiology* **511**, P. 8P.
- FOXDAL, P., BERGQVIST, Y., ECKERBLUM, S. & SANDHAGEN, B. (1992). Improving lactate analysis with the YSI 2300 GL: Hemolyzing blood samples makes results comparable with those for deproteinized whole blood. *Clinical Chemistry* **38**, 2110–2114.
- GAITANOS, G. C., WILLIAMS, C., BOOBIS, L. H. & BROOKS, S. (1993). Human muscle metabolism during intermittent maximal exercise. *Journal of Applied Physiology* **75**, 712–719.
- GERBINO, G., WARD, S. & WHIPP, B. (1996). Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *Journal of Applied Physiology* **80**, 99–107.
- GONZÁLEZ-ALONSO, J., QUISTORFF, B., KRUSTRUP, P., BANGSBO, J. & SALTIN, B. (2000). Heat production in human skeletal muscle at the onset of intense dynamic exercise. *Journal of Physiology* **524**, 603–615.
- HELLSTEN, Y., RICHTER, E. A., KIENS, B. & BANGSBO, J. (1999). AMP deamination and purine exchange in human skeletal muscle during and after intense exercise. *Journal of Physiology* **520**, 909–920.
- HILL, A. V. & WOLEDGE, R. C. (1962). An examination of absolute values in myothermic measurements. *Journal of Physiology* **162**, 311–333.
- HINCKLE, P. C. & YU, M. L. (1979). The phosphorous/oxygen ratio of mitochondrial oxidative phosphorylation. *Journal of Biological Chemistry* **254**, 2450–2455.
- KOGA, S., SHIOJIRI, T., KONDO, N. & BARSTOW, T. J. (1997). Effect of increased muscle temperature on oxygen uptake kinetics during exercise. *Journal of Applied Physiology* **83**, 1333–1338.
- KUSHMERICK, M. J., LARSON, R. E. & DAVIES, R. E. (1969). The chemical energetics of muscle contraction. I. Activation heat, heat of shortening and ATP utilization for activation-relaxation processes. *Proceedings of the Royal Society B* **174**, 293–313.
- LUTHANEN, P., RAHKILA, P., RUSKO, H. & VIITASALO (1987). Mechanical work and efficiency in ergometer bicycling at aerobic and anaerobic thresholds. *Acta Physiologica Scandinavica* **131**, 331–337.
- MACDONALD, M., PEDERSEN, P. K. & HUGHSON, R. L. (1997). Acceleration of VO₂ kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *Journal of Applied Physiology* **83**, 1318–1325.
- PAROLIN, M. L., CHESLEY, A., MATSON, M. P., SPRIET, L. L., JONES, N. L. & HEIGENHAUSER, J. F. (1999). Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *American Journal of Physiology* **277**, E890–900.
- POOLE, D. C., GAESSER, G. A., HOGAN, M. C., KNIGHT, D. R. & WAGNER, P. D. (1992). Pulmonary and leg VO₂ during submaximal exercise: implications for muscular efficiency. *Journal of Applied Physiology* **72**, 805–810.
- PUTMAN, C. T., JONES, N. L., LANDS, L. C., BRAGG, T. M., HOLLIDGE-HORVAT, M. G. & HEIGENHAUSER, J. F. (1995). Skeletal muscle pyruvate dehydrogenase activity during maximal exercise in humans. *American Journal of Physiology* **269**, E458–468.
- RASBAND, W. S. & BRIGHT, D. S. (1995). NIH Image: A public domain image processing program for the Macintosh. *Microbeam Analysis Society Journal* **4**, 137–149.
- SAUGEN, E. & VØLLESTAD, N. K. (1995). Non-linear relationship between heat production and force during isometric contractions in man. *Journal of Applied Physiology* **79**, 2043–2049.
- 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.
- VON DÖBELN, W. (1956). Human standard and maximal metabolic rate in relation to fat-free body mass. *Acta Physiologica Scandinavica* **37** (Supplementum 126), 1–79.
- WALSH, T. H. & WOLEDGE, R. C. (1970). Heat production and chemical change in tortoise muscle. *Journal of Physiology* **206**, 457–469.
- WILLIS, W. T. & JACKMAN, M. R. (1994). Mitochondrial function during heavy exercise. *Medicine and Science in Sports and Exercise* **26**, 1347–1354.
- WOLOSKER, H., ROCHA, J. B., ENGELENDER, S., PANIZZUTTI, R., DE MIRANDA, J. & MEIS, L. (1997). Sarco/endoplasmic reticulum Ca²⁺-ATPase isoforms: diverse responses to acidosis. *Biochemical Journal* **321**, 545–550.

Acknowledgements

We thank Merete Vannby, Ingelise Kring and Winnie Tagerup for excellent technical assistance. The study was supported by a grant from The Danish National Research Foundation (504-14). In addition, support was obtained from Team Danmark and The Sports Research Council (Idrættens Forskningsråd). J. González-Alonso was supported by a Marie Curie Research Training Grant (FMBICT950007).

Corresponding author

J. Bangsbo: Copenhagen Muscle Research Centre, Universitetsparken 13, DK-2100 Copenhagen Ø, Denmark.

Email: jbangsbo@aki.ku.dk