ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise

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Bangsbo, Jens, Peter Krstrup, José González-Alonso, and Bengt Saltin. ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. Am J Physiol Endocrinol Metab 280: E956–E964, 2001.—The aim of the present study was to examine whether ATP production increases and mechanical efficiency decreases during intense exercise and to evaluate how previous exercise affects ATP turnover during intense exercise. Six subjects performed two (EX1 and EX2) 3-min one-legged knee-extensor exercise bouts [66.2 ± 3.9 and 66.1 ± 3.9 (±SE) W] separated by a 6-min rest period. Anaerobic ATP production, estimated from net changes in metabolites like pyruvate and lactate, the anaerobic energy production of the biopsied muscle has been estimated in several studies that employ cycle exercise (13, 35, 37). It is, however, difficult from measurements on muscle biopsy material to determine the anaerobic energy turnover during whole body exercise such as cycling, because the mass and the activity of the muscles involved are unknown. Furthermore, the metabolic response of the biopsied muscle may not be representative of all of the muscles included in the exercise. Another problem is that the release of metabolites into the blood from the exercising muscles is often not taken into account when energy turnover is calculated, although this may represent a substantial contribution to the total energy production when the exercise lasts more than a few seconds (4).

In a few studies, the aerobic contribution to the energy turnover of the exercising muscles during intense and maximal cycle exercise has been determined through measurements of the pulmonary oxygen uptake (VO₂) (35, 37). However, such estimations require that the mass of the active muscles be known. Furthermore, it is assumed that the pulmonary VO₂ represents the VO₂ of the exercising muscle, but this assumption is not valid in the initial phase of intense exercise, as there is a time delay in the transport of blood from the exercising muscle to the lungs (9). The problems in determining the aerobic and anaerobic ATP and energy yield are minimized by using a knee-extensor exercise model in which the exercise is confined to the quadriceps muscle, allowing a rather precise determination of the mass of the active muscle (3, 4). Furthermore, by insertion of catheters in the femoral blood vessels, arterial as well as venous blood draining the exercising muscle can be collected, and blood flow to the exercising muscle can be measured. The model has been used to quantify the aerobic and anaerobic energy contribu-

MUCH KNOWLEDGE EXISTS about energy production during submaximal exercise, i.e., a work intensity lower than that eliciting maximum oxygen uptake (VO₂ max) (36). Less information has been obtained about ATP turnover and mechanical efficiency during supramaximal exercise. This may be due to the difficulties in quantifying the anaerobic energy production and determining oxygen utilization of the exercising muscles during this type of exercise.

To estimate the anaerobic energy production, muscle biopsies have been obtained before and after intense dynamic exercise. On the basis of the decrease in muscle ATP and creatine phosphate (CP), as well as accumulation of metabolites like pyruvate and lactate, the anaerobic energy production of the biopsied muscle has been estimated in several studies that employ cycle exercise (13, 35, 37). It is, however, difficult from measurements on muscle biopsy material to determine the anaerobic energy turnover during whole body exercise such as cycling, because the mass and the activity of the muscles involved are unknown. Furthermore, the metabolic response of the biopsied muscle may not be representative of all of the muscles included in the exercise. Another problem is that the release of metabolites into the blood from the exercising muscles is often not taken into account when energy turnover is calculated, although this may represent a substantial contribution to the total energy production when the exercise lasts more than a few seconds (4).

In a few studies, the aerobic contribution to the energy turnover of the exercising muscles during intense and maximal cycle exercise has been determined through measurements of the pulmonary oxygen uptake (VO₂) (35, 37). However, such estimations require that the mass of the active muscles be known. Furthermore, it is assumed that the pulmonary VO₂ represents the VO₂ of the exercising muscle, but this assumption is not valid in the initial phase of intense exercise, as there is a time delay in the transport of blood from the exercising muscle to the lungs (9). The problems in determining the aerobic and anaerobic ATP and energy yield are minimized by using a knee-extensor exercise model in which the exercise is confined to the quadriceps muscle, allowing a rather precise determination of the mass of the active muscle (3, 4). Furthermore, by insertion of catheters in the femoral blood vessels, arterial as well as venous blood draining the exercising muscle can be collected, and blood flow to the exercising muscle can be measured. The model has been used to quantify the aerobic and anaerobic energy contribu-

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tion during an intense exercise bout (6). However, little information exists about the muscle metabolic changes and $V_{O2}$ at the onset of exercise. In a recent study it was observed that heat production increased during intense knee-extensor exercise (24), which in part may be related to differences in the efficiency of the metabolic reactions involved in the exercise (46). However, it is not clear to what extent the various metabolic pathways are contributing to ATP turnover throughout the exercise and whether part of the increase in heat production is due to an increase in the rate of ATP production during intense exercise.

Various studies have focused on energy turnover during repeated maximal exercise, and it has been suggested that the energy turnover per work unit is lowered when intense exercise is repeated (22, 41). However, in these studies the aerobic energy turnover was not determined, and 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 with a constant exercise intensity, it has been observed that, when a second exercise bout was performed after both a 2.5-min and a 60-min rest period, the anaerobic energy production per work unit was significantly reduced without a change in leg $V_{O2}$ (7, 8). However, in these studies, the muscle $V_{O2}$ in the initial phase of exercise could not be determined accurately because of the limited number of measurements.

Thus the aim of the present study was to examine whether ATP turnover increases and mechanical efficiency decreases during supramaximal concentric exercise and to evaluate whether previous exercise altered the ATP production and mechanical efficiency. Subjects performed a 3-min intense knee-extensor exercise bout at a supramaximal work rate, which was repeated after a 6-min rest period. Arterial and femoral venous blood samples were collected, and leg blood flow was measured frequently during the exercise. In addition, a muscle biopsy was taken before and immediately after the exercise and on a separate occasion also twice in the initial phase of exercise.

SUBJECTS AND METHODS

Subjects

Six healthy male subjects ranging in age from 21 to 24 yr, with an average height of 178 (range: 172–183) cm and an average body mass of 72.5 (67.9–78.3) kg, participated in the experiment. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their informed consent to participate. The study was approved by the Frederiksberg, Copenhagen Ethics Committee.

Methods

During 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 (2). Before the experiment, the subjects had practiced the exercise on more than three separate occasions.

The subjects had a light breakfast ~3 h before an experiment, and they reported to the laboratory ~2 h before the experiment. With the subject in the supine position, a catheter was placed in a femoral artery under local anesthesia. The tip was positioned 1–2 cm proximal to the inguinal ligament. A catheter was also placed in the femoral vein of the experimental leg ~1–2 cm distal to the inguinal ligament. A thermistor for measurement of venous blood temperature was inserted through the catheter and was advanced 8–10 cm proximal to the tip.

After ~1 h of rest, the subject performed two 3-min knee-extensor exercise bouts (EX1 and EX2) with the experimental leg [66.2 ± 3.9 and 66.1 ± 3.9 (±SE) W; kicking frequency: 60.7 ± 2.0 and 60.6 ± 2.0 kicks/min] separated by a 6-min rest period (Fig. 1). Before each of the exercise bouts, the leg was passively moved for 5 s to accelerate the flywheel to obtain a constant power output from onset of exercise. After 60 min of rest, the entire exercise protocol was repeated with the same leg to allow for early measurements of thigh blood flow (EX3 and EX4).

Blood was drawn from the femoral artery ~10 and 5 s before as well as 2, 5, 10, 15, 40, 65, 90, 115, 150, and 170 s during EX1 and EX2. Femoral venous blood was collected ~15 and 5 s before as well as 2, 6, 9, 14, 29, 59, 89, 113, and 167 s during EX1 and EX2. Femoral venous blood flow was measured by the thermodilution technique (3) approximately every 30 s after ~1 min of EX1 and EX2, as well as 3 s before and 3, 7, 29, 34, 61, 66, 92, and 162 s during EX3 and EX4 performed after 1 h of rest.

Values of blood flow obtained at the same time during the first two exercise bouts (EX1 and EX2) agreed with the corresponding values of the two bouts after 60 min of rest (EX3 and EX4) [100 s: 4.83 ± 0.46 (EX1) vs. 5.13 ± 0.44 (EX3) l/min; 150 s: 5.63 ± 0.70 (EX1) vs. 5.39 ± 0.58 l/min (EX3); 100 s: 5.19 ± 0.64 (EX2) vs. 5.38 ± 0.59 (EX4) l/min; 150 s: 5.51 ± 0.63 (EX2) vs. 5.71 ± 0.61 (EX4) l/min]. Thus blood flow values after the 60-min rest period were used in the calculations. An occlusion cuff placed just below the knee was inflated (220 mmHg) during the exercises to avoid contribution of blood from the lower leg. Before and immediately after each of the exercise bouts, a biopsy was obtained from the m. vastus lateralis. On a separate occasion, the subjects performed the same knee-extensor exercise as in the main experiment for 5 and 15 s, i.e., 5 and 15 kicks, separated by a 45-min rest period, and a muscle biopsy was obtained at rest and after each of the exercise bouts.

Blood analysis. Oxygen saturation of blood and hemoglobin concentration were determined spectrophotometrically (Radiometer OSM-3 hemoximeter). The hemoximeter was calibrated spectrophotometrically by the cyanomethemoglobin method (18). Hematocrit (Hct) determinations were made in triplicate by use of microcentrifugation. A part of the blood sample was immediately placed in ice-cold water and centrifuged rapidly for 30 s. Then the plasma was collected and stored at −80°C until analyzed for pyruvate using a fluorometric assay (34).

Muscle sampling and analysis. Muscle biopsies were immediately frozen in liquid N$_2$ and stored at −80°C. The frozen muscle samples were weighed before and after freeze-drying to determine water content. The freeze-dried sample was dissected free of blood and connective tissue and extracted in a solution of 0.6 M perchloric acid (PCA) and 1 mM...
EDTA, neutralized to pH 7.0 with 2.2 M KHCO₃, and stored at -80°C until analyzed for CP and lactate by fluorometric assays (34).

Muscle mass. The mass of quadriceps femoris muscles was estimated by use of magnetic resonance imaging. Briefly, for each subject, 30–33 parallel axial T1-weighed images (seconds) of the right thigh were obtained with a multi-slice spin-echo FLASH sequence (repetition time 5500 ms, echo time 515 ms) by use of a body coil. Slice thickness was 3 mm, with a 12-mm interslice gap. Pixel size was 1.2 mm². This setting was selected to optimize image quality to clearly separate muscle, bone, fat, and connective tissue. Image analysis was performed using NIH Image software. The mean knee-extensor mass of the experimental leg was 2.35 kg, with a range of 1.94–2.79 kg.

Calculations. VO₂ and lactate and pyruvate release by the thigh were calculated by multiplying the blood flow or, for pyruvate, the plasma flow, by the difference between the femoral venous and arterial (v-adiff) concentrations, with blood transit time taken into account (see the next paragraph). A continuous blood flow curve was constructed for each subject by linear connection of the consecutive data points to obtain time-matched values of blood flow with the blood variables.

In the initial phase of exercise, VO₂ and metabolite exchange rise progressively; therefore, it is essential to know the blood transit time from the arteriole to the collection point of the venous blood samples to obtain comparable arterial and venous measurements. Therefore, to determine the transit time of the collection site in the femoral vein, four of the subjects carried out the experimental protocol on a separate occasion. The experimental conditions, including positioning of the catheters, were the same as in the main experiment. Before and frequently during EX1 and EX2, 2 mg of indocyanine green (ICG, Becton-Dickinson) in a concentration of 5 mg/ml were injected rapidly into the femoral artery, immediately followed by a flush with isotonic saline (5 ml). Blood was withdrawn from the femoral vein, and densitometer output was sampled with a computer. The time from injection to the time the curve peaked, corrected by transit time of the catheters (the dead space of the catheters divided by the pump flow), was used as the mean transit time (MTT). MTT (n = 4) was 10.8 ± 0.5, 7.0 ± 0.5, 6.4 ± 0.4, and 6.4 ± 0.5 s after 5, 25, 65, 152 s of EX1, respectively, and was 9.5 ± 0.7, 7.3 ± 0.5, 6.4 ± 0.3, and 6.1 ± 0.3 s, respectively, in EX2.

To determine the blood transit time from the femoral artery to the capillaries, ICG concentration was determined at three positions of the quadriceps muscle with a NIRO300 (Hamamatsu Photonics) with dual-channel near infrared (NIR) laser diodes. It was observed that this time accounted for about one-third of the MTT at various time points during exercise. When this value and the MTT for each individual were used, the average time that the collected artery and venous blood represented capillary blood was estimated. Average values were used for the two subjects for whom MTT was not determined. All blood variables are presented in relation to mean time at the capillary level.

Anaerobic ATP production of the quadriceps muscle for the time periods 0–5, 5–15, and 15–180 s was calculated as:

Fig. 1. Schematic representation of the experimental design. A: on 1 day subjects performed four 3-min intense knee-extensor exercise bouts at 66 W (EX1, EX2, EX3, and EX4). Femoral arterial and venous blood samples, as well as measurements of thigh blood flow and mean transit time, were collected. In addition, muscle biopsies were obtained before and after the first 2 exercise bouts. B: on a separate day, subjects performed a 5-s and a 15-s knee-extensor exercise bout (66 W), separated by 45 min of rest. A muscle biopsy was taken before and after the 5-s bout, as well as after the 15-s bout.
of lactate release was higher \( (P < 0.05) \) than in EX2 \( (P < 0.05) \). In EX2, the release of lactate was higher \( (P < 0.05) \) for the first 9 s compared with EX1, whereas after 60 s and toward the end of exercise, the release was lower \( (P > 0.05) \) in EX1 and the same in EX2 (Fig. 3). The total amount of lactate released in EX1 was higher \( (P < 0.05) \) than in EX2 (Fig. 2B).

Thigh blood flow was \( 0.17 \pm 0.09 \) l/min immediately before EX1, and it increased rapidly during exercise, reaching \( 3.85 \pm 0.35 \) l/min after 29 s and \( 5.39 \pm 0.82 \) l/min at the end of the exercise. Before EX2, thigh blood flow \( (3.13 \pm 0.33 \) l/min) was higher \( (P < 0.05) \) than before EX1, and it remained higher \( (P < 0.05) \) until 165 s of exercise.

Estimated net release of lactate (blood flow \( \times v_{qdiff} \)) was significant after 14 s \( (1.7 \) mmol/min), and it increased \( (P < 0.05) \) to 12.7 mmol/min during exercise (Fig. 3). The mean rate of lactate efflux was lower \( (P < 0.05) \) during the first 5 s and next 10 s than during the remaining 165 s of exercise (Fig. 2B). In EX2, the release of lactate was higher \( (P < 0.05) \) during the first 9 s compared with EX1, whereas after 60 s and toward the end of exercise, the release was lower \( (P > 0.05) \) in EX1 and the same in EX2 (Fig. 3). The total amount of lactate released in EX1 was higher \( (P < 0.05) \) than in EX2 (Fig. 2B).

The rate of lactate production by the quadriceps muscle, determined as the sum of the rates of lactate release and lactate accumulation, was not different in the various phases of exercise (Fig. 2B). The mean rate of lactate production was lower \( (P < 0.05) \) during EX2 than during EX1 (Fig. 2B).
A significant (P < 0.05) net release of pyruvate was observed after 14 s of EX1 (45 ± 13 μmol/min), and it peaked after 59 s at 183 ± 35 μmol/min. The mean rate of pyruvate release was lower (P < 0.05) during the first 5 s and after 10 s than during the rest of EX1 (−6 ± 8 and 24 ± 10 vs. 141 ± 26 μmol/min). In EX2, a release (P < 0.05) was observed before and during the first 6 s, but after 14 s and throughout the rest of exercise, pyruvate release was less than in EX2 than in EX1. The total release of pyruvate was less (P < 0.05) in EX2 than in EX1 (0.39 ± 0.07 vs. 0.17 ± 0.05 mmol).

The rate of CP utilization during the first 5 s, next 10 s, and remaining 165 s corresponded to rates of ATP production of 1.5 ± 0.6, 1.1 ± 0.4, and 0.2 ± 0.1 mmol ATP·kg dry wt−1·s−1, respectively (Fig. 4). For the same time periods, lactate production corresponded to rates of ATP production of 1.4 ± 0.7, 0.7 ± 0.3, and 1.0 ± 0.1 mmol ATP·kg dry wt−1·s−1, respectively (Fig. 4). By adding ATP production associated with pyruvate release and other sources (see METHODS), the rate of total anaerobic ATP production was estimated. It was higher (P < 0.05) during 0–5 and 5–15 s than during the rest of EX1 (Fig. 4). The mean rate of anaerobic ATP production was less (P < 0.05) in EX1 than in EX2 (1.2 ± 0.1 vs. 1.6 ± 0.1 mmol ATP·kg dry wt−1·s−1).

**Aerobic Energy Production**

Thigh \( \dot{V_O}_2 \) increased (P < 0.05) from 0.03 l/min immediately before EX1 to 0.13 l/min after 9 s, reaching 0.79 l/min at the end of EX1, respectively (Fig. 5). The mean rate of \( \dot{V_O}_2 \) during the first 5 s was less (P < 0.05) than during the next 10 s, which was smaller (P < 0.05) than during the remaining 165 s of exercise (0.10 ± 0.01 and 0.19 ± 0.03 vs. 0.63 ± 0.06 l/min), leading to corresponding differences in aerobic rate of ATP production (Fig. 4). In EX2, thigh \( \dot{V_O}_2 \) was higher (P < 0.05) than in EX1 during the first 119 s of exercise, whereafter no differences were observed. The mean rates of \( \dot{V_O}_2 \) and aerobic ATP production by the thigh were higher (P < 0.05) in EX2 than in EX1 (0.69 ± 0.08 vs. 0.59 ± 0.06 l/min and 5.1 ± 0.5 vs. 4.4 ± 0.3 mmol ATP·kg dry wt−1·s−1).

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**Fig. 3.** Thigh lactate release during EX1 (●) and EX2 (○). Values are means ± SE. *Significantly (P < 0.05) different from EX1.

**Fig. 4.** Rate of muscle ATP turnover (mmol ATP·kg dry wt−1·s−1) during 0–5, 5–15, and 15–180 s of EX1 as well as during EX1 and EX2, estimated as the sum of muscle anaerobic energy production determined as energy release related to utilization of CP (hatched part of bar), net lactate production determined as the sum of accumulation in muscle (open bar) and release to the blood (horizontally lined bar), net ATP utilization (vertically lined bar), others (crosshatched bar; see METHODS), and aerobic energy production (filled bar), determined from muscle oxygen uptake and estimated utilization of oxygen from myoglobin (see METHODS). Values are means ± SE. #Significantly (P < 0.05) different from 0–5 and 5–15 s.
between EX1 and EX2 (Fig. 6). The work output per ATP production was not different to EX2 (27 J mmol ATP), corresponding to a decrease in efficiency of 36%. Because the external work was constant, mechanical efficiency, expressed as work per ATP production, was significantly lower (P < 0.05) from 15 to 180 s than from 0 to 15 s of EX1 (25.0 ± 0.5 mmol ATP·kg dry wt−1·s−1; Fig. 4). The mean rate of ATP production was the same in EX1 and EX2 (6.0 ± 0.4 vs. 6.3 ± 0.5 mmol ATP·kg dry wt−1·s−1), but a greater (P < 0.05) fraction was provided from anaerobic sources during EX1 than during EX2 (27 ± 3 vs. 20 ± 1%; Fig. 5). Thus the average work output per ATP production was not different between EX1 and EX2 (Fig. 6).

The mean rate of ATP production (determined as the sum of the rates of anaerobic and aerobic ATP production) was 4.2 ± 1.2 and 3.8 ± 0.7 mmol ATP·kg dry wt−1·s−1 during the first 5 s and next 10 s, which were lower (P < 0.05) than during the remaining 165 s of EX1 (6.1 ± 0.3 mmol ATP·kg dry wt−1·s−1; Fig. 4). The mean rate of ATP production during the same time periods of EX2 was 5.1 ± 0.5 mmol ATP·kg dry wt−1·s−1, which is 39 to 25 J/mmol ATP. It should also be noted that the value for the last phase of exercise is of the same magnitude as the value found in animal studies using isolated muscle [for examples, 13–24 J/mmol ATP (17); 27 J/mmol ATP (33)].

The question is what causes the change in ATP production per work unit during concentric exercise. On the basis of in vitro studies on isolated muscles, it has been suggested that factors such as lowered pH, as well as elevated temperature and increased P:\(\text{Pi}\) levels, lower mechanical efficiency of contracting muscles through an increase in both Ca\(^{2+}\)-ATPase and myosin-ATPase activity (12, 15, 16, 47).

In the present study, muscle lactate increased progressively during the intense exercise, leading to an estimated decrease in pH from 7.1 to ~6.7 (29), which may have affected mechanical efficiency. In several human studies, it has been observed that VO\(_2\) progressively increases when submaximal exercise is contin-

**Fig. 5.** Thigh oxygen uptake during EX1 (●) and EX2 (○). Values are means ± SE. *Significantly (P < 0.05) different from EX1.

**ATP Production and Mechanical Efficiency**

The mean rate of ATP production (determined as the sum of the rates of anaerobic and aerobic ATP production) was 4.2 ± 1.2 and 3.8 ± 0.7 mmol ATP·kg dry wt−1·s−1 during the first 5 s and next 10 s, which were lower (P < 0.05) than during the remaining 165 s of EX1 (6.1 ± 0.3 mmol ATP·kg dry wt−1·s−1; Fig. 4). The mean rate of ATP production was the same in EX1 and EX2 (6.0 ± 0.4 vs. 6.3 ± 0.5 mmol ATP·kg dry wt−1·s−1), but a greater (P < 0.05) fraction was provided from anaerobic sources during EX1 than during EX2 (27 ± 3 vs. 20 ± 1%; Fig. 5). Thus the average work output per ATP production was not different between EX1 and EX2 (Fig. 6).

**DISCUSSION**

The present data show that the rate of ATP turnover increases during intense exercise performed at a constant work rate. Thus mechanical efficiency declines as intense exercise is continued. Furthermore, when intense exercise is repeated within some minutes, there is a shift toward greater aerobic energy contribution, but the total ATP production and mechanical efficiency are not significantly altered.

**ATP Production During Intense Exercise**

It was observed that ATP utilization during a constant load exercise was ~55% higher during the last phase of exercise compared with the first 15 s, which resulted in a decrease in the mechanical efficiency from 39 to 25 J/mmol ATP. It should, however, be considered how reasonable the estimations of ATP turnover are. The most critical assumption in the calculations is the P:\O\(_2\), which on the basis of in vitro studies on rat liver mitochondria has been suggested to be 2.5 (28). It should be noted that, if the in vivo P:\O\(_2\) had been as unrealistically low as 0.9, there would not have been a difference between the first 15 s of exercise and the remainder of exercise. Likewise, a fall in P:\O\(_2\) from 2.5 at the start to 1.2 at the end of exercise would be required to make the work performed per ATP produced constant throughout the exercise. However, even though there are indications from in vitro studies that mitochondrial respiration can be partly uncoupled at an increased intercellular Ca\(^{2+}\) concentration (25), it is unlikely that P:\O\(_2\) changes that much. This notion is supported by the observation that P:\O\(_2\) determined in mitochondria isolated from a human muscle biopsy obtained immediately after intense exhaustive exercise was the same as at rest (U. F. Rasmussen, P. Krustrup, J. Bangsbo, and H. N. Rasmussen, unpublished observation). The contribution of oxygen released from Mb is also uncertain, but because the assumed 50% reduction in Mb\(_2\)O\(_2\) utilized in the present study may be in the high range (43), the difference in ATP turnover between the first and later phases of exercise would be even greater if the Mb\(_2\)O\(_2\) utilized was actually smaller. Together, these considerations suggest that it is reasonable to conclude that the ATP production per work unit is lower in the first period of exercise. It should also be noted that the value for the last phase of exercise is of the same magnitude as the value found in animal studies using isolated muscle [for examples, 13–24 J/mmol ATP (17); 27 J/mmol ATP (33)].

The question is what causes the change in ATP production per work unit during concentric exercise. On the basis of in vitro studies on isolated muscles, it has been suggested that factors such as lowered pH, as well as elevated temperature and increased P:\(\text{Pi}\) levels, lower mechanical efficiency of contracting muscles through an increase in both Ca\(^{2+}\)-ATPase and myosin-ATPase activity (12, 15, 16, 47).

In the present study, muscle lactate increased progressively during the intense exercise, leading to an estimated decrease in pH from 7.1 to ~6.7 (29), which may have affected mechanical efficiency. In several human studies, it has been observed that VO\(_2\) progressively increases when submaximal exercise is contin-

**Fig. 6.** Muscle mechanical efficiency, determined as work performed per mmol of ATP produced during 0–5, 5–15, and 15–180 s of EX1 as well as during EX1 and EX2. Values are means ± SE. *Significantly (P < 0.05) different from 0–5 and 5–15 s.
used, but only at intensities leading to a significant accumulation of lactate in the blood (23), and it has been suggested that lowered muscle pH elevates $V_{O_2}$ and thus decreases mechanical efficiency. However, the finding of the same ATP turnover during EX1 and EX2, even though muscle pH probably was lower in the initial phase of EX2 (7), does indicate that any effect of lowered muscle pH is small. In accordance, in a previous study, the rate of ATP turnover was found to be the same whether intense knee-extensor exercise was preceded without or with intense arm exercise, with the latter condition leading to a further reduction of muscle pH (10). However, in that study and during EX2 in the present study, muscle pH may only during a minor fraction of the exercise have been lower than in EX1; therefore, it cannot be excluded that pH has an effect on ATP turnover and mechanical efficiency during intense exercise.

In separate experiments, it was observed that the muscle temperature increased from ~34.5 to 36.0°C during intense exercise at an intensity similar to that used in the present study. It is possible that the increase in temperature affected the mechanical efficiency, because, among other effects, it has been shown in vitro to decrease P/O (45), causing an increase in $V_{O_2}$ solely to maintain the rate of ATP production. During submaximal cycle exercise at a frequency of 60 rpm, it has been observed that $V_{O_2}$ was higher when muscle temperature was elevated by prior passive heating (20). However, in that study the difference was only ~5%, suggesting that any effect of temperature was small, which is supported by the fact that total ATP turnover and mechanical efficiency were the same during EX1 and EX2 in the present study, although the mean temperature was ~0.5°C higher in EX2 (data not shown). An increase in ATP turnover during the exercise may also be related to a lowering of molar free energy in ATP hydrolysis ($\Delta G$) caused by increases in metabolites like ADP and $P_i$, as well as lowering of pH and increasing temperature. It is, however, unclear whether a lowering of $\Delta G$ will change the work done per ATP split (46).

An increase in the total muscle ATPase activity as exercise progressed may have been due to recruitment of more fibers or more ATP-consuming fibers, i.e., fibers that have a high ATP utilization per force produced. However, it is not established whether there is a difference in efficiency between the various fiber types during concentric contractions in vivo. In in vitro studies, with use of either isolated muscles or single muscle fibers, it is observed that the efficiency of the different fiber types is closely related to the velocity, pattern, and type of contraction (11, 12, 14, 17). At low contraction velocities, the efficiency of slow-twitch (ST) fibers is higher than for fast-twitch (FT) fibers, whereas it appears to be the opposite at high speeds (11). In the present study, the quadriceps muscle was contracting at an average speed of ~135%/s, corresponding to ~20% of maximal velocity (1), which has been suggested to be within the optimum speed of ST fibers and nonoptimal for FT fibers (11, 17). Thus the higher ATP utilization during the last phase of exercise may be explained by a greater recruitment of FT fibers. However, further studies are needed to examine fiber type recruitment and efficiency of the different fibers during intense exercise.

**ATP Production During Repeated Intense Exercise**

When the intense exercise was repeated, muscle $V_{O_2}$ was elevated in the initial phase of exercise, which was associated with a higher activation state of pyruvate dehydrogenase (data not reported), but it is unclear whether this caused the greater muscle respiration. On the other hand, in accordance with a number of other studies (7, 8), muscle lactate production was reduced during the second exercise bout. Thus ATP production and mechanical efficiency were the same when intense exercise was repeated. It cannot be excluded, however, that a difference existed during the exercise. In an additional experiment, a 4-min exercise bout was performed twice, with bouts separated by 2 min of rest. Biopsies were obtained before and after 20 s of each exercise bout. It was observed that CP degradation during the first 20 s was the same for the first and second exercise bouts (1.8 ± 0.2 and 1.7 ± 0.2 mmol·kg dry wt $^{-1}·s^{-1}$), whereas muscle lactate accumulation was less ($P < 0.05$) in the second exercise bout (1.0 ± 0.2 vs. 0.3 ± 0.3 mmol·kg dry wt $^{-1}·s^{-1}$). Combining these data with those for $V_{O_2}$ and lactate/pyruvate release in the present study, the rate of ATP production during the first 20 s was the same during the two exercise bouts [4.2 (EX1) vs. 4.0 (EX2) mmol·kg dry wt $^{-1}·s^{-1}$]. These findings suggest that the rate of ATP turnover was the same in EX1 and EX2 in both the initial and the later phases of exercise. In other studies when repeated knee-extensor exercise was used, mechanical efficiency was observed to be unaltered, as in the present study, when exercise was repeated after a 2.5-min rest period but elevated when the rest period

![Fig. 7. Muscle mechanical efficiency, determined as work per total energy production during 0–15 and 15–180 s of an intense knee-extensor exercise, in which total energy production was determined from metabolic measurements (open bars, present study) and as the sum of total heat production and work performed (filled bars; Ref. 24). Values are means ± SE. #Significantly ($P < 0.05$) different from 0–15 s.](http://ajpendo.physiology.org/)
was 60 min (7, 8). The latter observation may be explained by the longer duration of the first exercise bout compared with the second bout (3.7 vs. 3.0 min), because the present study suggests that ATP turnover increases as exercise progresses, but it could also be related to the longer rest period between the exercise bouts compared with the present study. It should be noted that studies using repeated intense cycle exercise or isolated contracting muscles have suggested that mechanical efficiency is higher when exercise is repeated with a recovery period of <4 min (22, 41). However, in these studies the metabolic response of the exercising muscles could not be accurately determined and related to the work performed.

Energy Production During Intense Exercise

On the basis of net change in reactant levels of the metabolic reactions, it is possible to estimate the total energy production by use of average values of energy produced in each of the reactions determined in vitro. A molar enthalpy change (ΔH) value of 55 kJ/mol ATP for the net CP breakdown (43) and 67 kJ/mol ATP for glycolysis, with glycogen assumed as substrate (16), may be used (the corresponding value for oxidative phosphorylation is 95 kJ/mol ATP for a P/O of 2.5). Muscle aerobic contribution can be converted to energy production by use of a caloric value of 21.2 kJ/l O2 (38), if we assume that only pyruvate was oxidized. This assumption seems reasonable, because no glucose uptake was observed (data not reported), and fat oxidation during this type of exercise appears to be negligible (7). The mean rate of energy production can then be estimated to be 149 ± 29 and 275 ± 23 J/s during the first 15 and the remaining 165 s of exercise, respectively, corresponding to an increase (P < 0.05) in the energy production per work unit of ~85%. These values correspond to a decrease (P < 0.05) in mechanical efficiency, expressed as the ratio of work rate to the rate of energy production, from 57.9 ± 6.6% in the first 15 s to an average of 30.8 ± 1.6% during the remainder of the intense exercise. The observation that the rate of energy turnover is smaller in the initial phase of concentric exercise is in agreement with findings in another knee-extensor exercise study in which the rate of muscle energy turnover was estimated from heat production and power output (24). The rate of heat production during intense dynamic knee-extensor exercise doubled over 3 min of intense exercise, with one-half of the increase occurring in the first 40 s of exercise. The mean rate of energy turnover was 164 ± 12 J/s, and mechanical efficiency was 50.2 ± 5.8% during the first 15 s, which is similar to the values obtained in the present study (Fig. 7). During the remaining 165 s, the mean rate of energy turnover was somewhat lower and the mechanical efficiency higher than in the present study (Fig. 7). Nevertheless, with two independent methods, it has been demonstrated that energy turnover increases, and thus mechanical efficiency decreases, during intense exercise.

Since Krogh and Lindhard (32) in the early part of the last century determined the oxygen deficit as the difference between energy demand and V02, oxygen deficit has been frequently used as a measure of the anaerobic energy production (30, 35). The calculations have been based on the assumptions that the relation between energy turnover and power output is linear from moderate submaximal to supramaximal exercise, i.e., a work output higher than the intensity eliciting V02max, and that the energy turnover is constant during intense exercise. The first assumption has been questioned (4), and the present study demonstrates that the latter assumption is not valid. Apparently, in the initial phase of intense exercise, the mechanical efficiency of the exercising muscle is higher than during steady-state submaximal exercise, whereas as the intense exercise progresses, it may be lower compared with submaximal exercise. This means that the oxygen deficit determined in the traditional way overestimates the anaerobic energy turnover if the exercise time is short (<2 min). Thus, for exhaustive intense exercise, the magnitude of the true oxygen deficit depends on the duration and intensity of the exercise. This can also explain the observation of an agreement between the oxygen deficit and the anaerobic energy production determined from muscle and blood metabolic measurements for an exhaustive knee-extensor exercise bout lasting 3.2 min (6). It may be that the magnitude of overestimation of the true anaerobic energy turnover by the oxygen deficit method in the first phase of the exercise corresponded to the underestimation in the last phase of exercise.

Summary

The present data show that ATP production increases and mechanical efficiency decreases during intense exercise at a constant intensity, which may in part be due to a change in fiber type recruitment, an elevated temperature, and a lowered pH. However, further studies are needed to clarify the cause of the increase in ATP production per work unit during intense exercise and its functional importance. When intense exercise is repeated, the total ATP turnover is not changed if the exercise duration is the same.

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