Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise

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Bogdanis, Gregory C., Mary E. Nevill, Leslie H. Boobis, and Henryk K. A. Lakomy. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J. Appl. Physiol.* 80(3): 876–884, 1996.—This study examined the contribution of phosphocreatine (PCr) and aerobic metabolism during repeated bouts of sprint exercise. Eight male subjects performed two cycle ergometer sprints separated by 4 min of recovery during two separate main trials. *Sprint 1* lasted 30 s during both main trials, whereas *sprint 2* lasted either 10 or 30 s. Muscle biopsies were obtained at rest, immediately after the first 30-s sprint, after 3.8 min of recovery, and after the second 10- and 30-s sprints. At the end of *sprint 1,* PCr was 16.9 ± 1.4% of the resting value, and muscle pH dropped to 6.69 ± 0.02. After 3.8 min of recovery, muscle pH remained unchanged (6.80 ± 0.03), but PCr was resynthesized to 78.7 ± 3.3% of the resting value. PCr during *sprint 2* was almost completely utilized in the first 10 s and remained unchanged thereafter. High correlations were found between the percentage of PCr resynthesis and the percentage recovery of power output and pedaling speed during the initial 10 s of sprint 2 ($r = 0.84$, $P < 0.05$ and $r = 0.91$, $P < 0.01$). The anaerobic ATP turnover, as calculated from changes in ATP, PCr, and lactate, was 235 ± 9 mmol/kg dry muscle during the first sprint but was decreased to 139 ± 7 mmol/kg dry muscle during the second 30-s sprint, mainly as a result of a ~45% decrease in glycolysis. Despite this ~41% reduction in anaerobic energy, the total work done during the second 30-s sprint was reduced by only ~18%. This mismatch between anaerobic energy release and power output during *sprint 2* was partly compensated for by an increased contribution of aerobic metabolism, as calculated from the increase in oxygen uptake during *sprint 2* (2.68 ± 0.10 vs. 3.17 ± 0.13 l/min; *sprint 1* vs. *sprint 2;* $P < 0.01$). These data suggest that aerobic metabolism provides a significant part (~49%) of the energy during the second sprint, whereas PCr availability is important for high power output during the initial 10 s.

sprinting; muscle fatigue; recovery; glycogenolysis; glycolysis; muscle lactate; muscle pH; oxygen uptake

IN RECENT YEARS, physiologists have used the model of maximal dynamic exercise to further understanding of the regulation of the metabolic pathways and to shed light on the etiology of fatigue during high-intensity exercise. After the introduction of the Wingate test (2), it was possible to examine the metabolic responses to sprinting while concurrently monitoring the power output during the test. It is now well documented that during a single 30-s cycle ergometer sprint 25–30% of the ATP resynthesized from anaerobic metabolism comes from phosphocreatine (PCr) breakdown, while the major part (65–70%) comes from glycolysis (3, 32). However, such studies examining a single sprint can only make a limited contribution to understanding the causes of fatigue and limitations of performance, as all metabolic changes occur concurrently. More recently, the model of intermittent exercise has become more popular. This model has the advantage that, because of the different rates of recovery of various muscle metabolites, the metabolic status of the muscle can be changed and the effect on performance in a subsequent sprint can be examined (3, 7, 18, 27). With the use of this model, some authors have shown a great reduction in the rate of glycolysis in subsequent bouts of exercise without a proportional decrease in power output (7, 18, 27) and have suggested, although oxygen uptake ($\dot{V}O_2$) was not determined, that the decrease in energy supply from anaerobic pathways may be partially compensated by an increase in aerobic metabolism (18, 27). Thus one of the purposes of the present study was to examine whether $\dot{V}O_2$ is increased in a second bout of sprint exercise.

It has also been suggested that PCr resynthesis, and thus PCr availability, is important for power output recovery (7, 11, 25), but in most cases these suggestions have been based on speculation and not on simultaneous measurements of muscle metabolites. In a recent study (3), the time course of power output recovery in the first few seconds of a second 30-s sprint was found to occur in parallel with the resynthesis of PCr, despite muscle pH remaining low throughout recovery. However, muscle metabolism during the second sprint was not examined. Therefore, a second purpose of the present study was to examine the relationship between the metabolic status of the muscle before a second sprint and the subsequent performance and changes in muscle metabolites during that sprint.

Finally, although it is widely accepted by the sporting community that a high maximum oxygen uptake ($\dot{V}O_{2max}$) and endurance fitness (training status) are important determinants of the ability to recover power output after sprinting, there is little information to support such beliefs in the physiology literature. A further purpose of the present study was, therefore, to examine the relationship between $\dot{V}O_{2max}$, endurance fitness, and the recovery of power output during sprinting.

In summary, the purpose of the present study was to examine the changes in muscle metabolism and power output when a bout of sprint exercise was repeated after a short recovery interval to further understanding of the regulation of the metabolic pathways, the etiology of fatigue during sprinting, and the factors affect-

ing recovery from maximal exercise. Muscle metabolism during *sprint 2* was examined in more detail by taking muscle biopsies after the first 10-s and at the end of the second 30-s sprint. The study tests the following hypotheses: *1*) that an increase in aerobic metabolism partially compensates for the reduction in energy supply from anaerobic pathways during *sprint 2; 2*) that PCr is completely utilized during the first 10 s of *sprint 2* and that the recovery of power output during the first 10 s of the *sprint 2* is related to the PCr resynthesis after *sprint 1;* and *3*) that the recovery of power output in *sprint 2* is related to $\dot{V}O_{2max}$ and endurance fitness.

METHODS

Subjects. Eight male university students, aged 24 ± 2 (SD) yr, 177 ± 7 cm in height, and 79 ± 10 kg in body mass, volunteered to take part in this study. All subjects were physically active (recreational athletes) and were informed in writing about the purpose of the study, any known risks, and the right to terminate participation at will. Each expressed understanding by signing a statement of informed consent. A medical history questionnaire was also completed in the presence of the experimenter, and subjects with medical problems were excluded. The protocol was approved by the Ethical Committee of Loughborough University.

Equipment. A modified friction-loaded cycle ergometer (Monark, model 864), interfaced with a microcomputer, was used to attain high-frequency logging of the flywheel angular velocity. The ergometer frame was mounted on a baseplate and strengthened with metal struts to prevent bending of the frame and handle bars. The instantaneous power generated during the sprints was corrected for the changes in kinetic energy of the flywheel (14), and results were averaged over 1-s intervals. By taking into account the work done in accelerating the flywheel during the initial seconds of the sprint, peak power was always reached before peak speed. A restraining harness, passed around the subject's waist and fixed to a metal rail bolted on the floor behind the bicycle frame, restricted exercise to the leg muscles during the sprints. Toe clips were used with the subject's feet strapped to the pedals with strong tape.

Preliminary tests. During a preliminary visit, the $\dot{V}O_{2max}$ of each subject was determined by using a continuous incremental test on the Monark cycle ergometer. Subjects performed a 2-min warm-up at 60 W [60 revolutions/min (rpm)], and then power output was increased by 30–60 W every 3 min until exhaustion (~11–14 min). Power output at $\dot{V}O_{2max}$ was 303 ± 13 W. Expired air was collected during the last 60 s of each stage by using the Douglas bag technique. Samples of expired air were analyzed for fractions of O_2 and CO_2 by using a paramagnetic oxygen analyzer (Servomex-Sybron/Taylor, model 570A) and a carbon dioxide analyzer (Lira infrared gas analyzer, model 303), and the volume of expired air was measured by a Harvard dry-gas meter. All gas volumes were corrected to STPD.

In a separate session, subjects performed five continuous 4-min stages of submaximal cycling at power outputs corresponding to 61 ± 2, 71 ± 2, 80 ± 2, 86 ± 2, and 93 ± 2% $\dot{V}O_{2max}$. Expired air was collected during the last minute of each stage, and duplicate samples of arterialized capillary blood (20 µl each) were taken from a prewarmed thumb during the last 15 s of each stage for lactate (La) determination. From this test, the relative intensity (%$\dot{V}O_{2max}$) corresponding to a blood La concentration of 4 mmol/l (%4 mM) was estimated for each subject by linear interpolation. This value (%4 mM) was accepted as an indication of endurance training status (endurance fitness), as the %$\dot{V}O_{2max}$ at a given blood La concentration has been shown to increase with endurance training (12) and to be related to endurance capacity, as reflected by the cycling time to exhaustion at 88% $\dot{V}O_{2max}$ (6).

Main tests. Subjects were familiarized with sprint cycling by completing at least 3–4 separate sprint practice sessions, each at least 2 days apart. The two randomly assigned main tests were performed at the same time of day, 1 wk apart. Subjects recorded their diet (with estimated portion sizes) and refrained from intense physical exercise for 48 h before the first main trial. They repeated this regimen in the 48 h before *test 2.* A standardized warm-up consisting of 4 min of pedaling at 60 W, followed by one bout of 30 s at 80 W and one bout of 30 s at 100 W, preceded each test. This warm-up has been previously shown to cause a minimal metabolic disturbance (4). Five minutes after the completion of the warm-up, subjects performed two maximal cycle ergometer sprints, separated by 4 min of passive recovery on the bicycle seat. During one main trial, a 30-s sprint was followed by another 30-s sprint (30-30 main trial), and in the other main trial, a 30-s sprint was followed by a 10-s sprint (30-10 main trial; Fig. 1). The resistive load was 75 N·kN body wt^{-1} (average load: 59 ± 3 N), and each sprint started from a rolling start of ~70 rpm. Strong verbal encouragement was given during each sprint.

The following performance parameters were obtained for each sprint: peak power output (PPO); pedaling speed at which peak power output was attained (Sp_{PPO}); maximum pedaling speed (Sp_{max}); mean power output for the first 10 s (MPO_{10}), the last 20 s (MPO_{L20}), and the whole sprint (MPO_{30}); and the percent decline from peak to end power output (fatigue index; FI). The mean pedaling speed during the above time intervals was also calculated (Sp_{10}, Sp_{L20}, Sp_{30}). Expired air samples were collected in Douglas bags during each sprint. The test-retest reliability for $\dot{V}O_2$ measurements during sprinting was determined in separate experiments. For a 30-s sprint, *test 1* vs. *test 2* $\dot{V}O_2$ values were 2.53 ± 0.28 vs. 2.59 ± 0.29 l/min [not significant (NS) n = 19], and the correlation coefficient r was 0.94 (SE of estimate: 0.12 l/min). For a 10-s sprint, *test 1* vs. *test 2* $\dot{V}O_2$ values were 1.64 ± 0.20 vs. 1.69 ± 0.19 l/min (NS; n = 7), and the r was 0.96 (SE of estimate: 0.15 l/min).

Muscle samples. On arrival at the laboratory, subjects rested on a couch for 30 min while small incisions were made through the skin and fascia over the vastus lateralis muscle (in both main trials, two incisions were made in the same leg ~3 cm apart; in one of the two main trials, another incision was made in the other leg) under local anesthesia (1% plain lidocaine). All postexercise biopsies were taken with the subject sitting on the cycle ergometer (Fig. 1). The biopsy leg (left or right), the choice of the two or three sampling points in each main trial (from rest, after *sprint 1,* 3.8 ± 0.01 min into recovery, after the 10-s *sprint 2,* and after the 30-s *sprint 2*), and the main trial order (30-30 main trial or 30-10 main trial) were randomized in a balanced design, so that biopsies before and immediately after each sprint were taken from the same leg. Thus, over two main trials, a total of five biopsies were taken from each subject through different incisions in the skin. Muscle samples were plunged directly into liquid nitrogen in the needle and were kept in liquid nitrogen until they were freeze dried (within 24 h of sampling).

Analytical methods. The freeze-dried samples were dissected free of connective tissue and blood and homogenized. The muscle powder was then divided in two parts. One part of

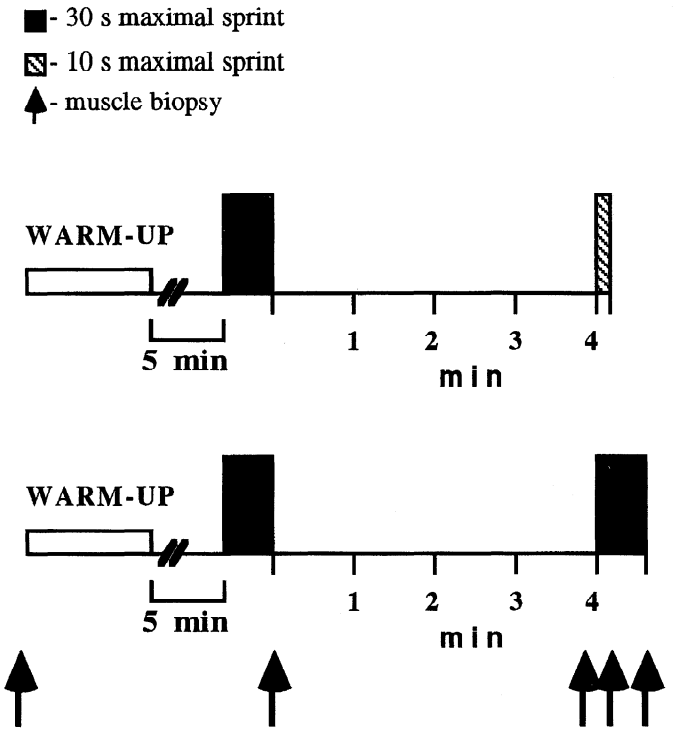

Fig. 1. Schematic representation of experimental design. A total of 5 muscle biopsies were obtained from each subject during the 2 conditions.

the powder was extracted with 0.5 mol/l $HClO_4$, and the extract was neutralized with 2.1 mol/l $KHCO_3$ (10). PCr, creatine (Cr), ATP, free glucose, glucose 1-phosphate (G-1-*P*), glucose 6-phosphate (G-6-*P*), fructose 6-phosphate (F-6-*P*), pyruvate (Pyr), and La were assayed enzymatically by fluorometric analysis (17). Glycogen was determined both on the neutralized extract (acid-soluble glycogen fraction) and on the muscle pellet left after the extraction procedure by prior HCl hydrolysis (acid-insoluble glycogen fraction). Muscle metabolite contents (except La and glucose) were adjusted to the individual mean total Cr content (PCr+Cr; range of corrections 0–11%). Because the sum PCr+Cr should not change during exercise, this acts as an internal reference to account for errors in muscle metabolite concentrations arising from the variable inclusion in the muscle samples of any remaining connective tissue, fat, or blood (10). All muscle metabolite concentrations are expressed as millimoles per kilogram dry muscle.

Muscle pH was determined on the second part of the freeze-dried muscle powder after homogenization at 4°C with a solution containing 145 mmol/l KCl, 10 mmol/l NaCl, and 5 mmol/l iodoacetic acid (23). The dilution ratio used was 100 μl of homogenizing solution per milligram dry muscle. Homogenates were equilibrated to 37°C for 5 min, and the pH was measured by using a MI-410 microelectrode (Microelectrode) connected to a radiometer acid-base analyzer (Radiometer PHM73).

Venous blood samples, taken from a cannula placed in an antecubital vein, were deproteinized in 2.5% perchloric acid and assayed for La by using an enzymatic and fluorometric method (17).

Calculations. Anaerobic ATP turnover (mmol/kg dry muscle) was calculated from the values of ATP, PCr, La, and Pyr before and immediately after each sprint, by using *Eq 1* below. The mean anaerobic ATP turnover rate (mmol·kg dry muscle^{-1}·s^{-1}) was obtained by dividing the anaerobic ATP turnover by the duration of the sprint (e.g., 10 or 30 s). No correction has been made for anaerobically produced ATP as a result of Pyr oxidation or for La efflux during the sprint.

$$\text{ATP turnover} = 2(-\Delta\text{ATP}) - \Delta\text{PCr} + 1.5\Delta\text{La} + 1.5\Delta\text{Pyr} \quad (1)$$

where 2 active phosphates are cleaved per ATP utilized and 1.5 mmol ATP is produced for every millimole of La and Pyr.

Glycogenolytic and glycolytic rates during each sprint (mmol glucosyl units·kg^{-1} dry muscle·s^{-1}) were calculated from accumulation of glycolytic metabolites

$$\text{glycogenolysis} = (\Delta\text{G-1-}P + \Delta\text{G-6-}P + \Delta\text{F-6-}P) + 0.5(\Delta\text{La} + \Delta\text{Pyr}) \quad (2)$$

$$\text{glycolysis} = 0.5(\Delta\text{La} + \Delta\text{Pyr}) \quad (3)$$

The underestimation in the above calculations, due to La efflux, is thought to be small because of the short duration of the exercise bouts and the slow kinetics for the release of La (time constant 30 min for repeated 30-s bouts of isokinetic cycling; see Ref. 16).

The concentration of P_i in the muscle after the sprints and during recovery was calculated from changes in ATP, PCr, and hexose monophosphates (G-1-*P*, G-6-*P*, and F-6-*P*)

$$P_i = 2.9 + [2(-\Delta\text{ATP}) - \Delta\text{PCr} - (\Delta\text{G-1-}P + \Delta\text{G-6-}P + \Delta\text{F-6-}P)]/3 \quad (4)$$

where 2.9 is resting value from Chasiotis (6); 3 is 3 liters of intracellular water per kilogram dry muscle; and P_i concentration is expressed in millimoles per liter of muscle water.

Statistical analysis. One-way (for muscle metabolites—factor: sampling point) or two-way analyses of variance (for performance variables) for repeated measures on both factors (sprint number and condition) were used where appropriate for statistical analysis (Statistica Mac). Where significant *F* ratios were found ($P < 0.05$), the means were compared by using a Tukey's post hoc test. Relationships between variables were examined by calculating the product moment correlation coefficient *r*. Results are presented as means ± SE.

RESULTS

Power output. The power output profiles during *sprint 1* and *sprint 2* are shown in Fig. 2. In both sprints, peak power was reached 2 s after the start of the sprint, whereas Sp_{max} was attained at 3.9 ± 0.2 s for *sprint 1* and at 4.0 ± 0.3 s for *sprint 2*. Reproducibility of PPO and MPO was checked by comparing mean values and calculating the correlation coefficients between the two main trials (*sprint 1* in the first main trial vs. *sprint 1* in the second main trial: PPO $r = 0.99$, MPO_{10} $r = 0.99$, MPO_{30} $r = 1.0$; NS between sprints. *Sprint 2:* PPO $r = 0.93$, MPO_{10} $r = 0.96$; NS between sprints). For example, PPO and MPO values (MPO_{30}) during *sprint 1* were 17.5 ± 1.2 and 9.2 ± 0.3 W/kg in the 30-30 main trial and 17.6 ± 1.1 and 9.2 ± 0.3 W/kg in the 30-10 main trial, respectively. Therefore, the mean values of the two main trials are presented for each sprint.

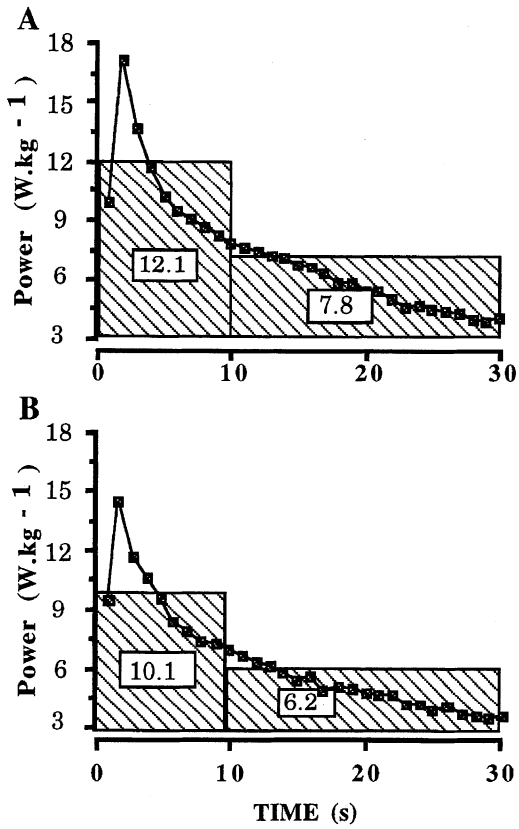

Fig. 2. Power output profiles (average for $n = 8$ subjects) for *sprint 1* (*A*) and *sprint 2* (*B*). Boxes represent mean power output for 1st 10 s and last 20 s of each sprint.

None of the power output indexes had returned to the control (*sprint 1*) values after the 4 min of passive recovery ($P < 0.01$). The MPO during the second 30-s sprint (MPO_{30}) was 7.6 ± 0.3 W/kg, which corresponded to 82 ± 2% of the MPO_{30} generated during *sprint 1*. PPO during *sprint 2* was 14.4 ± 0.8 W/kg (82 ± 2% of *sprint 1*), whereas MPO_{10} and MPO_{L20} were 84 ± 2 and 81 ± 2% of the corresponding *sprint 1* values, respectively (Fig. 2). As can be seen in Fig. 2, ~45% of the total work during the sprint was generated in the first 10 s, and this percent remained the same for both *sprint 1* and *sprint 2*. The FI was also the same for *sprint 1* and *sprint 2* (62 ± 3 vs. 62 ± 1%, NS). The pedaling speed parameters for both sprints are shown in Table 1.

The subjects with a higher PPO during *sprint 1* had a higher FI during that sprint ($r = 0.87$; $P < 0.01$) and a lower endurance fitness, as expressed by the $\%\dot{V}O_{2max}$ corresponding to a blood La concentration of 4 mmol/l (%4 mM; $r = -0.89$; $P < 0.01$). Furthermore, significant negative correlations ($r = -0.77$ to -0.81, $P < 0.05$ to $P < 0.01$) were found between power output during *sprint 1* (PPO and MPO_{10}) and the recovery of power (the power output during *sprint 2* expressed as a percentage of the power output during *sprint 1*) and pedaling speed during *sprint 2* ($\%MPO_{10}$, $\%Sp_{10}$). Finally, recovery of the above power and pedaling speed indexes during *sprint 2* was correlated with %4 mM ($r = 0.75$–0.94; $P < 0.05$ to $P < 0.01$).

Table 1. *Sp_{max}, Sp_{PPO}, Sp_{10}, Sp_{L20}, and Sp_{30} for sprint 1 and sprint 2*

	Sp_{max}	Sp_{PPO}	Sp_{10}	Sp_{L20}	Sp_{30}
Sprint 1	170 ± 7	156 ± 5	154 ± 5	111 ± 3	125 ± 4
*Sprint 2**	149 ± 5	138 ± 4	132 ± 3	90 ± 4	104 ± 3
% of *sprint 1*	88 ± 3	89 ± 2	86 ± 2	81 ± 2	83 ± 2

Values are means ± SE, $n = 8$ subjects. Sp_{max}, maximum pedaling speed; Sp_{PPO}, speed at which peak power output was attained; Sp_{10}, Sp_{L20}, and Sp_{30}, mean pedaling speed during 1st 10 s, last 20 s, and 30 s, respectively. Sp values in rpm. *All *sprint 2* parameters significantly different from *sprint 1*, $P < 0.01$.

Muscle metabolites. The muscle metabolite concentrations at rest, immediately after *sprint 1*, 12 ± 0.6 s before *sprint 2*, and after the second 10- and 30-s sprints are shown in Table 2. Total Cr was ~118 mmol/kg dry muscle and was similar at all sampling points. The decrease in muscle glycogen during *sprint 1* was ~99 mmol glucosyl units/kg dry muscle, compared with only 57 mmol glucosyl units/kg dry muscle during *sprint 2*. The soluble portion of glycogen represented 20–24% of the total at all sampling points. The major part (97%) of the decrease in glycogen during *sprint 1* could be accounted for by the accumulation of the measured glycolytic intermediates, Pyr, and La, whereas only 75% of the decrease in glycogen could be accounted for during *sprint 2*. The ~5% increase in muscle glycogen during the 3.8 min between sprints was not statistically significant. The rate of glycogenolysis and glycolysis during *sprint 2* was decreased by ~56% and ~45%, respectively, compared with *sprint 1*. The ratio of glycogenolysis to glycolysis was 1.74 ± 0.06 in the first 30-s sprint and 1.41 ± 0.09 and 1.38 ± 0.07, respectively, during the first 10-s and the whole 30-s period of *sprint 2*. The PCr content of the muscle ~6.6 s after *sprint 1* was 16.9 ± 1.4% of the resting value, and PCr was resynthesized to 78.7 ± 3.3% of the resting value by 3.8 min into the recovery (Fig. 3). During the first 10 s of *sprint 2*, PCr dropped rapidly to the post-*sprint 1* levels, with no significant decrease thereafter.

PCr levels at the end of the first sprint were similar in all subjects, but subjects with the highest PPO and MPO values in *sprint 1* had the slowest resynthesis of PCr. The percent resynthesis of PCr (%PCr) was negatively correlated with PPO ($r = -0.81$) and MPO during *sprint 1* (MPO_{10}, $r = -0.81$ and MPO_{30}, $r = -0.74$). Furthermore, %PCr resynthesis was closely correlated with %4 mM (Fig. 4), linking endurance fitness to PCr resynthesis. High correlations were found between the %PCr resynthesis and the percent recovery of MPO and mean pedaling speed during the first 10 s of *sprint 2* ($r = 0.84$, $P < 0.05$ and $r = 0.91$, $P < 0.01$).

There was a ~27% decrease in ATP immediately after *sprint 1*, but no further changes were seen after recovery or during the second sprint (Table 2). Calculated P_i concentration increased after *sprint 1* but had recovered considerably by 3.8 min after *sprint 1*. During the first 10 s of *sprint 2*, P_i increased to levels higher than after *sprint 1*, but no further change was seen at the end of 30 s. No correlation was found between P_i and power output recovery.

Table 2. *Muscle metabolites in vastus lateralis at rest, immediately after sprint 1, following 4 min of recovery, and after second 10- and 30-s sprint*

	Rest	*Sprint 1*	Recovery	*Sprint 2* (10 s)	*Sprint 2* (30 s)
Glycogen (total)	327.5 ± 14.3	228.3 ± 18.2[a]	240.5 ± 25.5[a]	223.5 ± 25.0[a]	183.5 ± 17.1[a,g]
PCr	75.2 ± 4.4	12.6 ± 1.2[a]	58.5 ± 2.3[a,b]	15.3 ± 1.4[a,c]	8.8 ± 2.5[a,c]
Cr	42.3 ± 2.1	105.2 ± 3.7[a]	58.6 ± 3.0[a,b]	102.2 ± 3.5[a,c]	108.8 ± 3.0[a,c]
P_i	2.9	16.2 ± 1.3[a]	6.4 ± 1.4[a,b]	20.1 ± 1.9[a,f,c]	20.4 ± 1.7[a,f,c]
ATP	27.0 ± 0.8	19.6 ± 0.9[a]	22.2 ± 1.0[a]	19.7 ± 1.3[a]	20.5 ± 1.2[a]
Glucose	1.7 ± 0.2	7.0 ± 0.4[a]	7.8 ± 0.5[a]	9.6 ± 1.2[a]	10.7 ± 1.0[a,f]
G-1-*P*	0.2 ± 0.1	3.1 ± 0.6[a]	0.8 ± 0.1[b]	1.1 ± 0.3[b]	1.3 ± 0.3[b]
G-6-*P*	1.4 ± 0.1	27.4 ± 1.3[a]	14.2 ± 0.7[a,b]	20.0 ± 0.8[a,b,c]	22.5 ± 1.4[a,b,c]
F-6-*P*	0.3 ± 0.1	9.6 ± 1.4[a]	2.7 ± 0.3[b]	3.8 ± 0.5[e,b,c]	5.1 ± 0.6[a,b,c]
Pyruvate	0.5 ± 0.1	4.1 ± 0.4[a]	1.1 ± 0.2[b]	2.0 ± 0.2[a,b]	2.4 ± 0.3[a,b,c]
Lactate	5.8 ± 0.9	108.0 ± 4.5[a]	72.7 ± 5.9[a,b]	106.6 ± 4.9[a,c]	129.3 ± 5.2[a,b,c,d]

Values are means ± SE for 7 subjects, expressed in mmol/kg dry muscle. Muscle glycogen expressed in mmol glucosyl units/kg dry muscle. P_i, calculated total inorganic phosphate (in mmol/liter muscle water). PCr, phosphocreatine; Cr, creatine; G-1-*P*, glucose 1-phosphate; G-6-*P*, glucose 6-phosphate; F-6-*P*, fructose 6-phosphate. Significant differences: [a]$P < 0.01$ from Rest; [b]$P < 0.01$ from *sprint 1;* [c]$P < 0.01$ from recovery; [d]$P < 0.01$ from *sprint 2* (10 s); [e]$P < 0.05$ from rest; [f]$P < 0.05$ from *sprint 1;* [g]$P < 0.05$ from recovery.

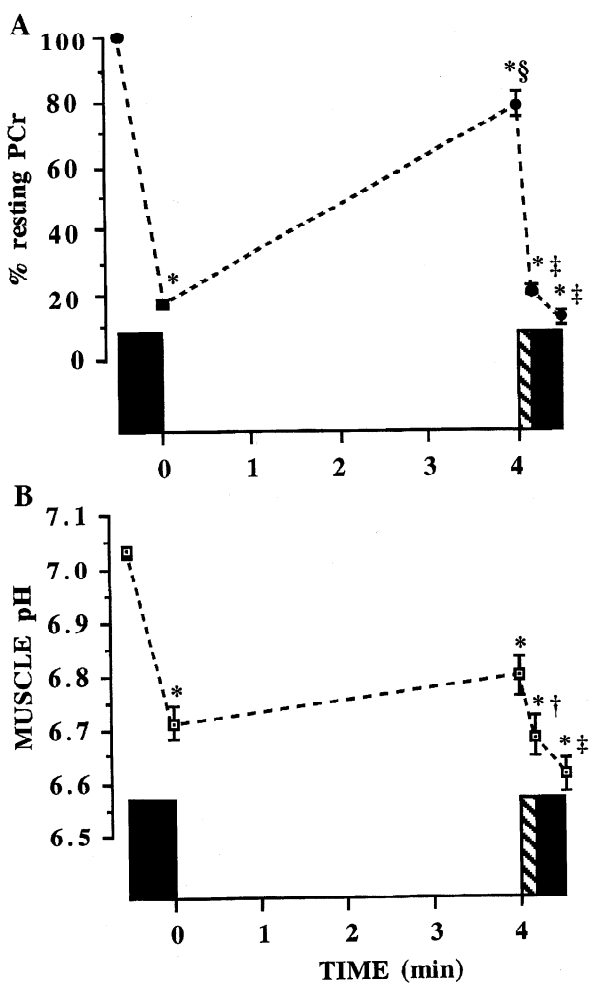

Fig. 3. Time course of changes in muscle phosphocreatine (PCr; *A*) and muscle pH (*B*) during 2 sprints separated by 4 min of passive recovery. *Sprint 1* was 30 s and *sprint 2* was either 10 or 30 s (sprints are represented by bars). Values for PCr are expressed as %resting value ($n = 7$ subjects). *$P < 0.01$ from resting value; †$P < 0.05$ and ‡$P < 0.01$ from pre-*sprint 2* value; and §$P < 0.01$ from post-*sprint 1* value.

A considerable proportion of P_i released during ATP and PCr breakdown appeared in the form of hexose monophosphates G-1-*P*, G-6-*P*, and F-6-*P*. The large increase of hexose monophosphates, especially G-6-*P*, after *sprint 1* was attenuated during the second sprint (Table 2).

Muscle La and pH. Muscle La increased to 108.0 ± 4.5 mmol/kg dry muscle immediately after *sprint 1,* and 67 ± 3% of that remained in the muscle at 3.8 min into the recovery period. Therefore, subjects started the second 30-s sprint with a high muscle La content, and the accumulation of La during that second 30-s sprint was decreased by 45%. The rate of La accumulation during the first 10 s of *sprint 2* was about threefold higher than that during the last 20 s of the same sprint.

Changes in muscle pH before, after, and during the recovery between the two sprints are shown in Fig. 3.

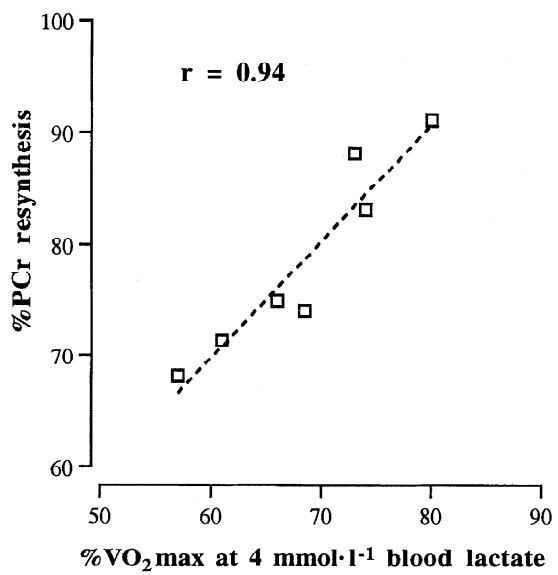

Fig. 4. Relationship between %maximal oxygen uptake ($\dot{V}O_{2max}$) corresponding to a blood lactate concentration of 4 mmol/l and %PCr resynthesis after 4 min of passive recovery following a maximal 30-s sprint ($n = 7$ subjects, $P < 0.01$).

Muscle pH decreased to 6.69 ± 0.02 immediately after *sprint 1* and only increased slightly to 6.80 ± 0.03 (NS) during recovery. During the second sprint, muscle pH was lower in comparison with the recovery value at 3.8 min at both 10 and 30 s (6.69 ± 0.03 at 10 s and 6.61 ± 0.03 after 30 s). No relationship was found between muscle pH before *sprint 2* and %PCr resynthesis or power output recovery.

Blood La. Blood La increased to ~9 mmol/l immediately after *sprint 1* and continued to increase during the recovery to reach ~12 mmol/l before *sprint 2.* There was no significant difference between blood La concentration immediately after the second 10- and 30-s sprint, but blood La was lower 3.5 min after the second 10-s sprint compared with 3.5 min after the second 30-s sprint (14.3 ± 0.7 vs. 16.0 ± 0.8 mmol/l, $P < 0.05$).

ATP turnover. The anaerobic ATP turnover, as calculated from changes in ATP, PCr, La, and Pyr, was 235 ± 9 mmol/kg dry muscle during *sprint 1* and was decreased to 139 ± 7 mmol/kg dry muscle during the second 30-s sprint. This ~41% reduction in the calculated anaerobic ATP turnover from *sprint 1* to *sprint 2* was more than twice as high as the ~18% decrease in MPO during *sprint 2.* However, the contribution of aerobic metabolism to energy supply was increased during *sprint 2,* as indicated by the increase in $\dot{V}O_2$ from 2.68 ± 0.10 l/min (61 ± 2% $\dot{V}O_{2max}$) during *sprint 1* to 3.17 ± 0.13 l/min (72 ± 3% $\dot{V}O_{2max}$) during *sprint 2* ($P < 0.01$). Ventilation was 110.0 ± 10 and 136.0 ± 7.7 l/min in *sprint 1* and *2,* respectively (*sprint 1* vs. *sprint 2,* $P < 0.01$). High correlations were found between $\dot{V}O_{2max}$ (in $ml \cdot kg^{-1} \cdot min^{-1}$) and the percent aerobic contribution to both *sprint 1* ($r = 0.79, P < 0.05$) and *sprint 2* ($r = 0.87, P < 0.01$).

The calculated anaerobic ATP turnover during the first 10 s and the last 20 s of *sprint 2* was 9.8 ± 0.8 and 2.1 ± 0.4 mmol·kg dry $muscle^{-1} \cdot s^{-1}$, respectively (a 78% decrease), whereas $\dot{V}O_2$ increased from 1.96 ± 0.11 (45 ± 2% $\dot{V}O_{2max}$) to 3.76 ± 0.12 l/min (85 ± 3%), respectively. The contribution of PCr during the first 10 s of *sprint 2* was high, amounting to ≈43% of the anaerobic energy supply. However, PCr concentration was very low at the end of the first 10 s of *sprint 2,* and its contribution dropped to only 15.6 ± 3.3% of anaerobic ATP turnover during the last 20 s of the second sprint. The rate of glycogenolysis during the first 10 s of *sprint 2* was positively correlated with the ATP turnover rate during the same time interval ($r = 0.88$, $P < 0.01$). During the last 20 s of *sprint 2,* glycolytic rate was reduced to only about one-third of that calculated for the first 10 s of that sprint.

DISCUSSION

This study was designed to examine changes in muscle metabolism during *sprint 2* and to relate these changes to the recovery of power output. There was a 41% reduction in the calculated anaerobic ATP turnover from *sprint 1* to *sprint 2,* mainly as a result of a 45% decrease in glycolysis (Fig. 5). However, MPO during *sprint 2* was only 18% lower than in *sprint 1.* One main finding of the present study was that this mismatch between anaerobic energy release and power output during *sprint 2* was partly compensated for by an increased contribution of aerobic metabolism as reflected by the increase in $\dot{V}O_2$ during the second sprint. The other main finding of the study was that PCr was almost completely broken down during the first 10 s of *sprint 2* and remained unchanged thereafter (Fig. 3). A relationship was found between the recovery of power output in the first 10 s of *sprint 2* and the resynthesis of PCr and between the recovery of power output and endurance fitness, as reflected by the percentage of $\dot{V}O_{2max}$ corresponding to a blood La concentration of 4 mmol/l (%4 mM) during submaximal exercise.

In the present study, the number of muscle biopsies was kept to an absolute minimum, which was thought to be consistent with a sound experimental design. Thus biopsies taken in only one of the main trials needed to be representative of muscle metabolism during both main trials. Such assumptions were considered to be safe, as there was excellent reproducibility of the performance variables during the two main trials, and the pre-main-trial regimen controlling both exercise and diet used in the present study has been shown

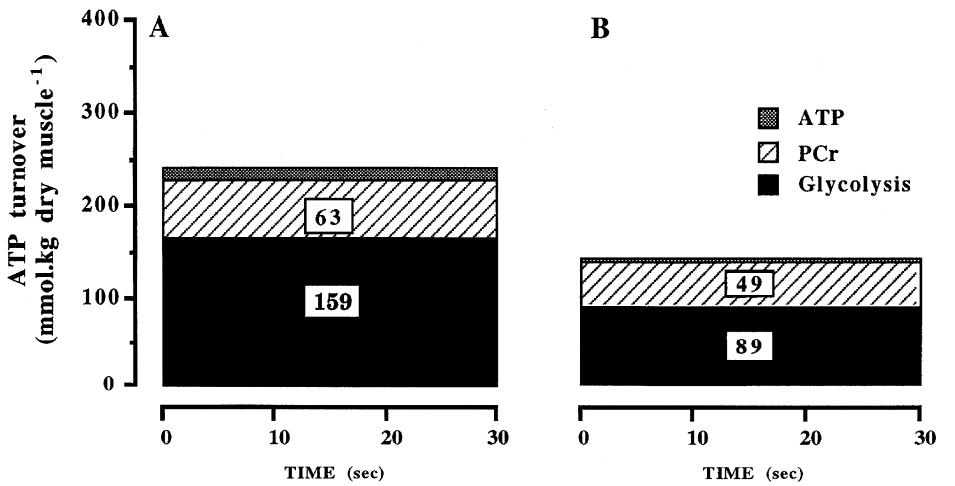

Fig. 5. Calculated ATP utilization during 2 30-s sprints (*A*: *sprint 1*; *B*: *sprint 2*) separated by 4 min of passive recovery. Boxes represent contribution of ATP-producing processes to total ATP utilization ($n = 7$ subjects).

to result in very similar resting muscle metabolites on two separate occasions 1 wk apart (30). In addition, no account was taken, in the calculations of ATP turnover, of the 12-s gap between the muscle biopsy before *sprint 2* and the start of the sprint. However, from our previous work (3) we know that the there is little recovery of muscle metabolites between 3 and 6 min after a single 30-s sprint. For example, from our model of PCr resynthesis we estimate that the change in PCr during the 12-s period before *sprint 2* in the present study will be <1 mmol/kg dry muscle. Thus any errors due the assumption that the metabolic status of the muscle at 12 s before *sprint 2* is representative of the metabolic status at the start of the sprint will be minimal.

The range of muscle metabolites in the present study and the additional information gained from the biopsy at 10 s into the second sprint have not been previously reported for repeated sprint cycling of 30-s duration, although the reduction in the glycolytic rate in the second sprint was of the same magnitude as that observed during isokinetic cycling (18, 27). All of the decrease in ATP occurred during the first 30-s sprint, so that, despite the loss of PCr during the first 10 s of *sprint 2* and the reduction in the glycolytic rate from the first 10 s to the last 20 s of *sprint 2,* there was no further loss of ATP. This lack of change in ATP in the second sprint is particularly surprising, as muscle pH failed to recover and had decreased further by 10 and 30 s of *sprint 2.* Under these conditions, an increase in AMP deaminase activity might have been expected. This continued decrease in pH, however, provides a possible explanation for the reduction in the glycolytic rate in the second 30-s sprint in comparison with the first 30-s sprint and in the last 20 s in comparison with the first 10 s of the second sprint, through an inhibitory effect on the key enzymes phosphorylase and phosphofructokinase.

PCr was almost entirely depleted during the first 10 s of *sprint 2,* and the large contribution of PCr to energy supply during this period probably supported the observed high power output. About 45% of the total work during the second 30-s sprint was generated in the first 10 s, and power output decreased at a rapid rate reaching 55% of peak power at 10 s (Fig. 2). During the last 20 s of *sprint 2,* power output declined at a lower rate, and the contribution of PCr was minimal. These observations would imply that the availability (amount) of PCr before a repeated sprint may be related to the ability to generate high power during the initial seconds of the sprint. This suggestion is supported by the high correlations found in the present study between the percentage of PCr resynthesis and the percentage restoration of MPO and speed during the initial 10 s of *sprint 2* ($r = 0.84$ and $r = 0.91$), whereas no such relationships were seen for power output during the last 20 s of the sprint. Further support for the significance of PCr during the initial seconds of repeated sprints comes from the results of a previous study, where the time course of PCr resynthesis after a 30-s sprint was found to be parallel with the time course of PPO restoration (3). Also two preliminary studies (15, 31) have shown that occlusion of the circulation to one leg during the 4-min recovery between repeated 30-s bouts of maximal isokinetic cycling prevents PCr resynthesis and reduces total work in the subsequent sprints by ~15% in the occluded in comparison with the nonoccluded leg. All of the observed decrease in work occurred during the first 10–15 s of the sprint, and no differences in muscle metabolites were found between the occluded leg and the leg with the free circulation, apart from the different PCr level before the second sprint. Similar evidence demonstrating the importance of PCr availability for muscle metabolism and power generation during repeated bouts of maximal exercise has been provided by studies in which muscle PCr content was increased after oral Cr supplementation. In one of these studies, ATP degradation was attenuated and power output recovery was increased, whereas there were no changes in muscle La accumulation during the second of two 30-s bouts of maximal isokinetic cycling separated by 4 min after, in comparison with before, oral Cr ingestion (9).

It is recognized that the relationship between power output recovery and PCr resynthesis may not be causal and that the recovery of power output may be influenced by many other factors (e.g., changes in pH, electrolytes, etc.). However, in the present study, power output recovery and changes in other metabolites (H^+, La, P_i, or $H_2PO_4^-$) were unrelated. The relationship between PCr and power output recovery may indicate, however, that when PCr is available, increases in muscle metabolites such as ADP (in the cell microenvironment), which may cause fatigue, are prevented. If this were the case, then PCr availability would be crucial for removing the products of ATP hydrolysis from all the cell adenosinetriphosphatases (myosin, Ca^{2+}, and Na^+-K^+) and thus it could have wide-ranging effects on cell function.

In the present study, both the resynthesis of PCr and the recovery of power output were related to endurance fitness (%4 mM), but no relationship was found between the resynthesis of PCr or power output and muscle pH. These results provide further support for earlier suggestions that muscle pH has little influence on PCr during the first few minutes of recovery and that oxygen supply to the mitochondria may be more important (24). Endurance-trained individuals (as reflected by %4 mM in the present study) are expected to have an increased capillary network, a high muscle oxidative capacity, and may, in addition, have a greater proportion of type I fibers (6, 28, 29). Support for the notion that endurance-trained individuals will have a faster rate of resynthesis of PCr comes from studies examining mainly isometric muscle actions, with the use of the noninvasive method of phosphorus magnetic resonance spectroscopy (^{31}P-MRS). These ^{31}P-MRS studies have provided evidence that PCr resynthesis is faster in endurance-trained athletes (34) and that it can be improved by endurance training (19). Furthermore, it has been reported that the PCr resynthesis rate is positively correlated with the activity of citrate

synthase (20), which in turn parallels capillary density (29), and also that the PCr resynthesis rate is reduced in patients with mitochondrial myopathies (22). A link has therefore been established between oxidative capacity and PCr resynthesis after exercising small muscle groups (e.g., calf muscles) by using noninvasive methods. The present study provides evidence showing this link following maximal sprint exercise, when PCr was directly determined by using chemical methods.

In addition to the relationship between endurance fitness and PCr/power output recovery, aerobic metabolism was found to contribute significantly to energy supply, especially during *sprint 2*. The increase in $\dot{V}O_2$ from *sprint 1* to *sprint 2* was ~18%. This finding is consistent with the previously reported increase in $\dot{V}O_2$ found between the first and fourth 1-min bouts of constant-velocity cycling performed at an exercise intensity of 120% $\dot{V}O_{2max}$ with a 4 min-rest interval between bouts (8). The findings of the present study are also consistent with the 13% (NS) increase in leg $\dot{V}O_2$ in the second bout of dynamic knee extensor exercise reported by Bangsbo and colleagues (1). Thus it seems likely that leg $\dot{V}O_2$ was increased in the second sprint in the present study. If it is assumed that the active muscle mass represented 20% of body mass [based on calculations (13) in subjects from the same population (33)], that all of that mass was equally active, and that the contribution from myoglobin and hemoglobin was the same in both sprints, it may be estimated (21) that the aerobic contribution to *sprint 1* and *2* was 34 ± 2 and 49 ± 2%, respectively. The value for *sprint 1* is similar to estimates reported in other studies in which either the oxygen-deficit method (28%; Ref. 32) or theoretical calculations in combination with power output data were used (28%; Ref. 26). Other authors have suggested a higher percent aerobic contribution (~40%, assuming 25% body mass as muscle mass) for 30 s of constant-velocity cycling (21). The difference in the percent aerobic contribution between that study and the present study may reflect a greater contribution by type I fibers at the slower pedal velocites during constant-velocity cycling in comparison with sprinting (90 rpm compared with peak values of 170 rpm in the present study). Although all of these estimates are subject to the errors inherent in the assumptions (for example, in the present study, if the active muscle mass was assumed to be 25% of body mass, the aerobic contribution would be 29 ± 2 and 44 ± 2%, respectively, for *sprint 1* and *2*), the findings of the present study do highlight the importance of the aerobic contribution to energy supply in sprint cycling, particularly in the second bout of exercise where $\dot{V}O_2$ reached 85% $\dot{V}O_{2max}$ during the last 20 s. The high correlations found between $\dot{V}O_{2max}$ and percent contribution by aerobic metabolism during *sprint 2* show the significance of a high aerobic power when performing repeated sprints. The increase in $\dot{V}O_2$ during *sprint 2* compensates, at least in part, for the apparent mismatch between the decrease in power output (18%) and the decrease in anaerobic energy supply (41%) from *sprint 1* to *2*. Any small remaining differences may be the result of an improvement in muscle efficiency from *sprint 1* to *2*, resulting from the reduction in pedal speed according to the parabolic shape of the power-velocity relationship.

In summary, this study has shown that (*1*) an increase in aerobic metabolism partially compensates for the reduction in energy supply from anaerobic pathways during *sprint 2* and that aerobic metabolism makes an important contribution to energy supply during repeated sprints; (*2*) that PCr is completely utilized during the first 10 s of *sprint 2* and that there is a relationship between power output recovery and PCr resynthesis but no relationship between power output recovery and any other metabolite measured in this study; and (*3*) that $\dot{V}O_{2max}$ and endurance fitness appear to be important, respectively, in determining the magnitude of the aerobic contribution to, and the recovery of power output during, repeated sprints.

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