9135 *Journal of Physiology* (1999), **519.3**, *pp.*901–910

In vivo ATP synthesis rates in single human muscles during high intensity exercise

Glenn Walter *, Krista Vandenborne †, Mark Elliott ‡ and John S. Leigh ‡

**Department of Physiology, † Department of Rehabilitation Medicine and ‡ Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104-6021, USA*

(Received 8 January 1999; accepted after revision 1 July 1999)

1. *In vivo* ATP synthesis rates were measured in the human medial gastrocnemius muscle during high intensity exercise using localized ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS). Six-second localized spectra were acquired during and following a 30 s maximal voluntary rate exercise using a magnetic resonance image-guided spectral localization technique.
2. During 30 s maximal voluntary rate exercise, ATPase fluxes were predominantly met by anaerobic ATP sources. Maximal *in vivo* glycogenolytic rates of 207 ± 48 mM ATP min^{-1} were obtained within 15 s, decreasing to 72 ± 34 mM ATP min^{-1} by the end of 30 s. In contrast, aerobic ATP synthesis rates achieved 85 ± 2% of their maximal capacity within 9 s and did not change throughout the exercise. The ratio of peak glycolytic ATP synthesis rate to maximal oxidative ATP synthesis was $2{\cdot}9 \pm 0{\cdot}9$.
3. The non-P_i, non-CO_2 buffer capacity was calculated to be $27{\cdot}0 \pm 6{\cdot}2$ slykes (millimoles acid added per unit change in pH). At the cessation of exercise, P_i, phosphomonoesters and CO_2 were predicted to account for $17{\cdot}2 \pm 1{\cdot}5$, $5{\cdot}57 \pm 0{\cdot}97$ and $2{\cdot}24 \pm 0{\cdot}34$ slykes of the total buffer capacity.
4. Over the approximately linear range of intracellular pH recovery following the post-exercise acidification, pH_i recovered at a rate of $0{\cdot}19 \pm 0{\cdot}03$ pH units min^{-1}. Proton transport capacity was determined to be $16{\cdot}4 \pm 4{\cdot}1$ mM (pH unit)$^{-1}$ min^{-1} and corresponded to a maximal proton efflux rate of $15{\cdot}3 \pm 2{\cdot}7$ mM min^{-1}.
5. These data support the observation that glycogenolytic and glycolytic rates are elevated *in vivo* in the presence of elevated P_i levels. The data do not support the hypothesis that glycogenolysis follows Michealis–Menten kinetics with an apparent K_m for [P_i] *in vivo*.
6. *In vivo*-measured ATP utilization rates and the initial dependence on PCr and glycolysis were similar to those previously reported in *in situ* studies involving short duration, high intensity exercise. This experimental approach presents a non-invasive, quantitative measure of peak glycolytic rates in human skeletal muscle.

Skeletal muscle has a high potential to generate ATP anaerobically. The power output achieved during 30 s of maximal sprinting drastically exceeds the power output necessary to elicit a maximal O_2 uptake (Q_{max}). If the only limitation to glycogenolytic ATP synthesis were glycogen levels, rates as high as 282 mM ATP min^{-1} ($\sim 4 \times Q_{max}$) could be sustained for 90 s in Type II fibres, as shown by *in situ* substrate utilization studies (Greenhaff *et al.* 1994; Casey *et al.* 1996). On the other end of the scale, subjects with enzyme deficiencies in glycogenolysis and glycolysis (Duboc *et al.* 1987) experience extensive PCr depletion without acidosis and are characterized by exercise intolerance (Duboc *et al.* 1987). Therefore, glycolytic rates must be large enough to provide sufficient oxidizable substrates to support mitochondrial ATPase synthesis, as well as to match the difference between the catalytic capacity of functional ATPases, Q_{max} and creatine kinase. This latter inter-relationship has been postulated to be fundamental to the muscle's phenotype (Greenhaff *et al.* 1994; Casey *et al.* 1996).

Both indirect and direct measurements of ATP provisions have demonstrated large transient increases in anaerobic ATP synthesis rates. Based on pulmonary O_2-deficit measurements, Bangsbo *et al.* (1990) demonstrated that during 30 s of high intensity exercise of an isolated muscle group (130% Q_{max}), anaerobic energy provision accounts for 80% of the total ATP synthesis. *In situ* measurements, which have the advantage that direct measurements of anaerobic provisions can be made in single muscles, have demonstrated that peak lactate accumulation rates can occur within 10 s of high intensity exercise, and dramatically decrease during the next 30 s (Boobis *et al.* 1982; Jacobs *et al.* 1983; Jones *et al.* 1985). However, due to the damaging

nature of the biopsy technique, *in situ* measurements are limited in temporal resolution and are poor for repeated measurements. MRS, on the other hand, provides a viable alternative for the serial measurement of changes in anaerobic ATP provisions during exercise. MRS has been successfully used to measure muscle glycogen (Chen *et al.* 1994), lactate (Pan *et al.* 1991), intracellular pH (pH_i) and phosphate kinetics (Walter *et al.* 1997). In addition, the development of ^{31}P-MRS localization methods now makes it possible to measure high energy phosphate and pH_i kinetics in single human muscles of different fibre type composition.

The relationship between lactate formation and changes in pH_i is known to depend on the physio-chemical buffering of the myoplasm (Sahlin, 1978; Hultman & Sahlin, 1980; Mainwood & Renaud, 1985). For instance, the extraction of lactate production rates from changes in pH_i depends on the correction for changes in proton concentration due to proton and/or lactate efflux (V_{eff}), the creatine kinase reaction, and the amount of protons buffered by muscle proteins (β_i) and bicarbonate (β_{CO_2}), in combination with the build-up of metabolites possessing pK_a values within the physiological range. *In situ* measurements of pH_i changes are often extrapolated based on the relationship between whole muscle homogenate pH and the sum of lactate and pyruvate concentrations (Sahlin, 1978; Hultman & Sahlin, 1980; Spriet, 1989). Unfortunately, this relationship is muscle specific (Spriet, 1989) and depends on the exercise conditions (Sahlin, 1978). Furthermore, this empirical relationship obscures the reported differences in buffer capacity (McKenna *et al.* 1996) and lactate/proton efflux rates (Kemp & Radda, 1994; Thompson *et al.* 1996; Juel, 1997).

In this study, *in vivo* anaerobic and aerobic ATP synthesis rates were measured in human skeletal muscle under exercise conditions known to elicit peak ATP utilization rates. The interplay between high energy phosphates and phosphorylation of sugars by the glycolytic pathway was examined in the context of maximal exercise conditions. *In vivo* muscle bioenergetics were studied in a single muscle (medial gastrocnemius) using localized ^{31}P-magnetic resonance spectroscopy.

METHODS

Subjects

The medial gastrocnemius muscle of 10 healthy subjects (range 19–37 years; eight males, two females) was studied. Eight of the subjects were moderately active, exercising at low intensity one to three times a week, whereas the remaining two were collegiate sprint runners. Six of the subjects had performed the in-magnet exercise protocol on previous occasions, while four of the subjects had never performed the exercise protocol. All subjects were informed of the purpose of the experiment and gave their written consent. The research protocol was approved by the Institutional Review Board of the University of Pennsylvania.

Experimental set-up

All studies were performed in a 1 m bore, 2·0 T superconducting magnet, interfaced with a home-built spectrometer (Walter *et al.* 1997). Subjects were placed in a supine position on an exercise bed, with their right leg suspended in a moulded cast to reduce motion artifacts and blood flow limitations. The subject's right foot was positioned on a non-magnetic, variable resistance calf ergometer built for in-magnet exercise. Exercise consisted of repeated maximal rate (2–3 Hz) plantar flexions (~30% maximum voluntary contraction (MVC)) over a 40 deg range of motion, with the knee extended (Walter *et al.* 1997). The high repetition rate was chosen to cause maximal or near-maximal muscle fibre recruitment. The exercise was performed for a total of 9 or 30 s. Straps around the shoulders, waist, knee and ankle were used to secure the body during exercise.

Magnetic resonance spectroscopy

Localized spectra were obtained from the medial gastrocnemius using an oblong surface coil in combination with a one-dimensional localization technique described previously (Walter *et al.* 1997). The surface coil was a single turn 6 cm × 8 cm oblong coil, double tuned to both ^{31}P and ^{1}H frequencies. The coil was placed over the upper one-third of the medial gastrocnemius. The volume of interest (VOI) was selected from multi-slice, transaxial, surface-coil proton images of the subject's calf acquired at the centre, proximal and distal ends of the coil as previously described (Walter *et al.* 1997). The typical size of the VOI selected within the medial gastrocnemius was ~2·7 cm × 5·0 cm × 7 cm (depth × width × length). All spectra were acquired with a sampling rate of 3000 Hz and 1024 complex data points. The pulse repetition time (TR) was set at 3 s, such that localized data were obtained in 6 s bins (two signal averages per time point). The rotating frame frequency was set between the P_i and PCr frequency to minimize chemical shift localization errors of the γ-ATP, P_i and PCr resonances. Spectra were enhanced using the nuclear Overhauser effect (NOE). The homogeneity of the magnetic field was optimized using the free proton signal.

Analysis of spectra

The NMR spectral data were filtered with an exponential filter corresponding to a line broadening of 5 Hz. The spectra were phased manually and the areas of the P_i, PCr and phosphomonoester (PME) peaks were determined by manual integration. PCr and ATP kinetics were determined by the complex principle component method (Elliott *et al.* 1999). Resting peak areas were corrected for saturation effects using fully relaxed localized spectra collected with a TR of 30 s. Absolute concentrations were calculated, based on a resting ATP concentration of 8·2 mM (Harris *et al.* 1974). This value has been reported for both human fast- and slow-twitch fibres (Jansson *et al.* 1987; Söderlund & Hultman, 1991). The inosine monophosphate (IMP) contribution to the PME pool was calculated based on changes in [ATP] (Jansson *et al.* 1987), such that the remaining PME levels primarily represented glucose 6-phosphate (G6P). Intracellular pH (pH_i) was calculated from the chemical shift of P_i based on the equation $pH_i = 6{\cdot}75 + \log(\delta - 3{\cdot}27)/(5{\cdot}69 - \delta)$, where δ is the chemical shift of the P_i peak in parts per million (p.p.m.), relative to PCr (Walter *et al.* 1997). Free ADP and AMP levels were determined using the near-equilibrium of creatine and adenylate kinase adjusted for changes in pH and a free [Mg^{2+}] of 1 mM (Golding *et al.* 1995).

Calculation of intracellular buffer capacity, proton efflux and ATP synthesis rates

***In vivo* buffer capacity.** The apparent buffer capacity (β_{tot}) in slykes (millimoles acid added per unit change in pH) was measured during a rest-to-work transition based on changes in pH_i and PCr. During the initial 6 s of exercise, changes in proton concentration due to anaerobic glycolysis (L), and proton efflux rates (V_{eff}) were

assumed to be negligible (Kemp & Radda, 1994) such that:

$$\beta_{tot} = \frac{mQ - (\theta \mathrm{d}PCr/\mathrm{d}t)}{\mathrm{d}pH/\mathrm{d}t}, \quad (1)$$

where the concentration of protons (mM) released by PCr, when coupled to P_i formation by functional ATPases (Wolfe *et al.* 1988), is represented by θ, and the factor m provides a minor correction due to the production of protons by aerobic metabolism (Q) (in mM ATP min^{-1}) (Mainwood & Renaud, 1985; Kemp & Radda, 1994). For calculation of Q see below.

$$m = 0{\cdot}16/(1 + 10^{(6{\cdot}1 - pH)}), \quad (2a)$$

$$\theta = 1/(1 + 10^{(pH - 6{\cdot}75)}). \quad (2b)$$

Due to the high concentration of P_i with a pK_a of 6·75 and the presence of glucose 6-phosphate (G6P) with a pK_a of 6·20, the contribution of each compound to the apparent buffering capacity throughout exercise can be calculated as a function of its concentration (mM) and the intracellular pH (pH_i):

$$\beta_{P_i} = \frac{2{\cdot}303[P_i]}{(1 + 10^{(pH_i - 6{\cdot}75)})(1 + 10^{(6{\cdot}75 - pH_i)})}, \quad (3a)$$

$$\beta_{G6P} = \frac{2{\cdot}303[G6P]}{(1 + 10^{(pH_i - 6{\cdot}20)})(1 + 10^{(6{\cdot}20 - pH_i)})}. \quad (3b)$$

During short duration, high intensity exercise, the muscle can be assumed to function as a closed system (Kemp & Radda, 1994), such that changes in bicarbonate due to interactions with arterial blood are small:

$$\beta_{CO_2} = \frac{2{\cdot}3 \times S \times P_{CO_2} \times 10^{(pH - 6{\cdot}1)}}{(1 + 10^{(pH_i - 6{\cdot}1)})(1 + 10^{(6{\cdot}1 - pH_i)})}, \quad (4)$$

where S is the solubility of carbon dioxide (1·3 mM kPa^{-1}) and P_{CO_2} is 5 kPa (Kemp & Radda, 1994). Therefore, the apparent buffering capacity of the muscle (β_{tot}) at any time point is a function of P_i, G6P and HCO_3^- concentrations and pH_i:

$$\beta_{tot} = \beta_i + \beta_{P_i} + \beta_{CO_2} + \beta_{G6P}. \quad (5)$$

The inherent buffer capacity (β_i) was calculated by subtracting out the contributions made by inorganic phosphate (β_{P_i}), G6P (β_{G6P}) and bicarbonate (β_{CO_2}) to β_{tot}:

$$\beta_i = \beta_{tot} - \beta_{P_i} - \beta_{CO_2} - \beta_{G6P}. \quad (6)$$

ATP synthesis rates. The glycolytic rate (L) (in mM ATP min^{-1}) was calculated based on changes in pH_i during high intensity exercise after correcting for the buffering of protons by changes in PCr, ATP, the rate of aerobic ATP utilization (Q), the apparent muscle buffer capacity (β_{tot}), and proton efflux (V_{eff}):

$$L = -\beta_{tot}(\mathrm{d}pH/\mathrm{d}t) - mQ - \theta(\mathrm{d}PCr/\mathrm{d}t) + V_{eff}. \quad (7)$$

The contribution of aerobic metabolism to changes in proton load was calculated as mQ. In the absence of oxygen limitations and changes in intramitochondrial redox, Q has been shown to be related to [ADP], [ADP][P_i]/[ATP] and [PCr] in skeletal muscle (Klingenberg, 1961; Leigh *et al.* 1986; Balaban, 1990). We chose to describe mitochondrial regulation under control of [ATP]/[ADP][P_i] (Klingenberg, 1961; Leigh *et al.* 1986). Q (mM ATP min^{-1}) was estimated throughout the exercise protocol as:

$$Q = \frac{Q_{max}}{1 + (K_{0{\cdot}5}/([ADP][P_i]/[ATP]))}, \quad (8)$$

where the Q_{max} (mM ATP min^{-1}) was determined using PCr resynthesis kinetics following 9 s of high intensity exercise (Paganini *et al.* 1997; Walter *et al.* 1997). We have previously shown that eqn (8) is well fitted with $K_{0{\cdot}5} = 0{\cdot}11$ mM in the human gastrocnemius muscle (Walter, 1997). The calculated value for Q was also experimentally verified by measuring initial PCr resynthesis rates at 9 and 30 s of exercise (Paganini *et al.* 1997; Walter, 1997).

Proton efflux rates. Proton efflux rates (V_{eff}; mM H^+ min^{-1}) were calculated throughout the recovery period using the observation that in the absence of muscle contraction glycolysis ceases (Quistorff *et al.* 1992), such that:

$$V_{eff} = \beta_{tot}(\mathrm{d}pH/\mathrm{d}t) + mQ + \theta(\mathrm{d}PCr/\mathrm{d}t). \quad (9)$$

In vivo pH_i and PCr recovery kinetics following 30 s of exercise were fitted to biexponential functions (Fig. 3) and used to determine dPCr/dt and dpH_i/dt. V_{eff} throughout the exercise was calculated based on the observation that lactate–proton co-efflux (mM min^{-1}) is linearly dependent on pH_i (Bangsbo *et al.* 1993; Kemp *et al.* 1994; Richardson *et al.* 1998; Newcomer *et al.* 1999):

$$V_{eff} = \lambda \Delta pH_i, \quad (10)$$

where λ (mM (pH unit)$^{-1}$ min^{-1}) was determined based on a least-squares fit of V_{eff} *vs.* ΔpH_i ($pH_{rest} - pH_{observed}$) during the recovery from the 30 s of exercise, starting after the post-exercise acidification phase (Fig. 3*C*). pH_i recovery rates (pH units min^{-1}) were also measured over the approximately linear region of the recovery.

RESULTS

P_i, PCr and pH kinetics during exercise

Changes in PCr, P_i and pH_i were determined with a temporal resolution of 6 s in the medial gastrocnemius. Basal PCr and P_i content and intracellular pH were 37·7 ± 2·8 mM, 4·09 ± 0·53 mM and 7·02 ± 0·01, respectively. At the end of 30 s of high intensity exercise, PCr and ATP levels were 7·68 ± 0·85% and 62·4 ± 5·3% of their basal levels. P_i increased to 32·2 ± 2·6 mM and PMEs to 14·7 ± 1·5 mM of which 3·09 ± 0·44 mM was attributed to IMP. Following an initial alkalization, pH_i decreased approximately linearly at a rate of 1·65 ± 0·12 pH units min^{-1} to 6·53 ± 0·04 at the end of 30 s (Fig. 1). ADP increased from 10·5 ± 2·6 μM at rest to 169 ± 40 μM at the end of exercise. AMP increased from 0·02 ± 0·01 μM at rest to 7·4 ± 3·8 μM at the cessation of exercise (Fig. 2).

Buffer capacity of human muscle

At the onset of exercise, a rapid drop in PCr levels of the medial gastrocnemius was matched by an increase of pH_i to 7·13 ± 0·02 within 6 s. Based on Δ[PCr]/Δ[pH_i] (137 ± 19 mM (pH unit)$^{-1}$) during the initial 6 s of exercise, the total medial gastrocnemius buffer capacity was 41·4 ± 6·6 slykes, where P_i, PMEs and CO_2 were predicted to account for 8·5 ± 1·5, 2·5 ± 1·0 and 4·03 ± 0·03 slykes of the β_{tot}, respectively. The non-P_i, non-CO_2 buffer capacity (β_i) was calculated to be 27·0 ± 6·2 slykes. At the cessation of exercise P_i, PMEs and CO_2 were calculated to account for 17·2 ± 1·5, 5·6 ± 1·0 and 2·24 ± 0·34 slykes of the β_{tot}. To determine the reproducibility of this measurement, β_i was determined at the onset (22·7 ± 3·7 slykes) and at the cessation (24·4 ± 6·1 slykes) of exercise in the medial gastrocnemius in a single subject during 13 separate trials.

Proton efflux rates in human muscle

Over the approximately linear range of pH_i recovery following the initial post-exercise acidification, pH_i recovered at a rate of 0·19 ± 0·03 pH units min^{-1}. Based on the result of the biexponential fits to both PCr and pH_i recoveries (Fig. 3), λ was determined to be 16·4 ± 4·1 mM (pH unit)$^{-1}$ min^{-1} and corresponded to a maximal efflux rate of 15·3 ± 2·7 mM min^{-1} following the attainment of a minimum pH_i of 6·18 ± 0·02 pH units. The reproducibility of the proton efflux measurements was determined in a single subject during eight high intensity exercise tests with an end exercise pH_i and minimum pH equal to 6·45 ± 0·07 and 6·23 ± 0·05 pH units, respectively. In this subject, intracellular pH recovered with an approximate linear rate of 0·13 ± 0·01 pH units min^{-1}. The maximal efflux rate and λ were calculated to be 6·33 ± 0·26 mM min^{-1} and 10·52 ± 1·25 mM (pH unit)$^{-1}$ min^{-1}.

Glycogenolytic and glycolytic flux

Glycogenolytic and glycolytic rates reached their peak at 15 s (Fig. 4). At 15 s of high intensity exercise, glycogenolysis was 113 ± 26 glucosyl units min^{-1} while the glycolytic rate was 35% less at 73·2 ± 16 glucosyl units min^{-1}. Glycogenolytic flux decreased at the end of exercise despite elevated AMP, ADP (Fig. 2), and P_i levels (Fig. 5).

Total ATP synthesis rates

The rate of ATP synthesis due to anaerobic glycolysis (*L*), aerobic metabolism (*Q*) and creatine kinase (CK) was calculated during the 30 s of high intensity exercise in the medial gastrocnemius and is displayed in Fig. 6. Note that

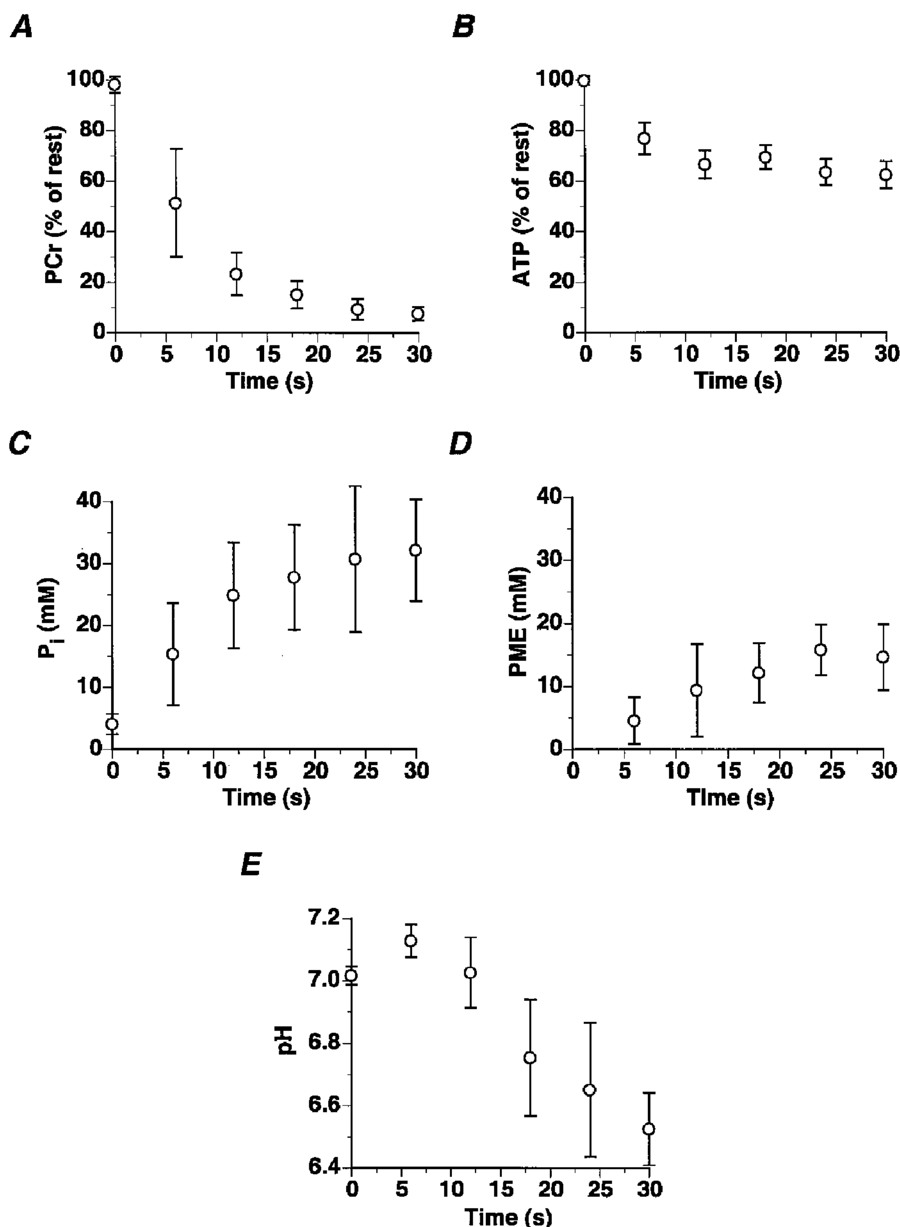

Figure 1

Changes in medial gastrocnemius PCr (*A*), ATP (*B*), P_i (*C*), PME (*D*) and intracellular pH (*E*) during 30 s of maximal rate exercise (values are means ± S.D.).

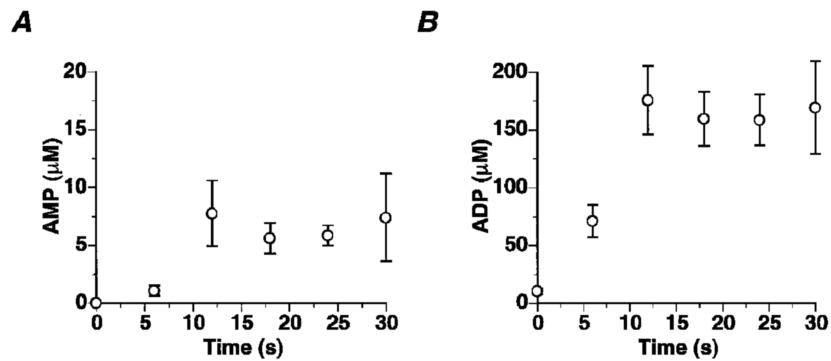

Figure 2

Changes in medial gastrocnemius AMP (*A*) and ADP (*B*) during 30 s of maximal rate exercise (data are means ± S.E.M.).

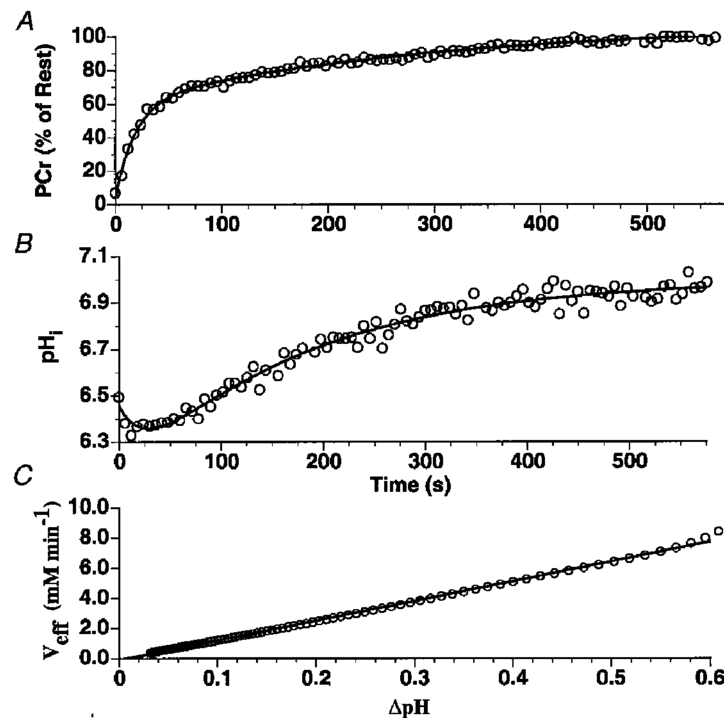

Figure 3. Typical PCr and intracellular pH (pH_i) kinetics as well as the relationship between V_{eff} and ΔpH during the recovery from 30 s of maximal rate exercise

The PCr (*A*) and pH_i (*B*) kinetics were used to determine PCr utilization rates, buffer capacity, proton efflux rates (V_{eff}; *C*), oxidative capacity, and glycolytic flux. PCr and pH recoveries were fitted as a sum of exponentials with amplitudes A_1 and A_2, and rate constants k_1 and k_2. PCr kinetics were fitted as:

$$y = [\text{Rest} - \text{Depl}][A_1(1 - e^{-tk_1}) + A_2(1 - e^{-tk_2})] + \text{Depl},$$

where Rest is the fully recovered PCr level and Depl is the initial PCr level. pH kinetics during recovery were fitted as:

$$y = [A_1 e^{-k_1 t} + A_2(1 - e^{-k_2 t})] + \text{pH}_{\text{min}},$$

where pH_{min} is the minimum pH obtained during recovery. Following a period of post-exercise acidification, pH_i recovered with an approximate linear rate of 0·13 pH units min^{-1} and with a rate constant of 0·34 min^{-1}. Based on the V_{eff} kinetics following the post-exercise acidification period (*C*) the peak efflux rate was 8·4 mM min^{-1} and λ was determined to be 13·1 mM (pH unit)$^{-1}$ min^{-1}.

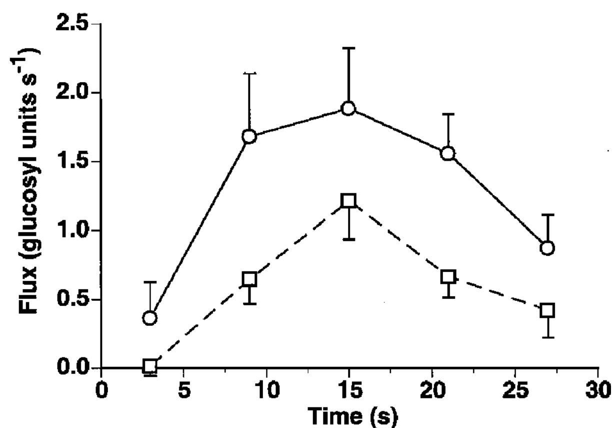

Figure 4

Glycogenolytic (○) and glycolytic (□) rates in the medial gastrocnemius during maximal rate exercise (data are means ± S.E.M.).

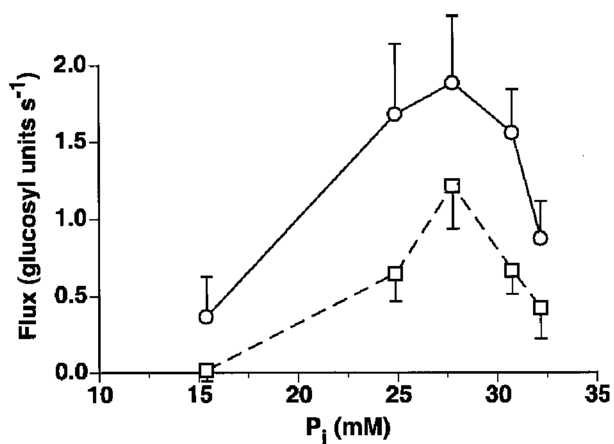

Figure 5

Glycogenolytic (○) and glycolytic (□) rates in the medial gastrocnemius as a function of P_i (data are means ± S.E.M.).

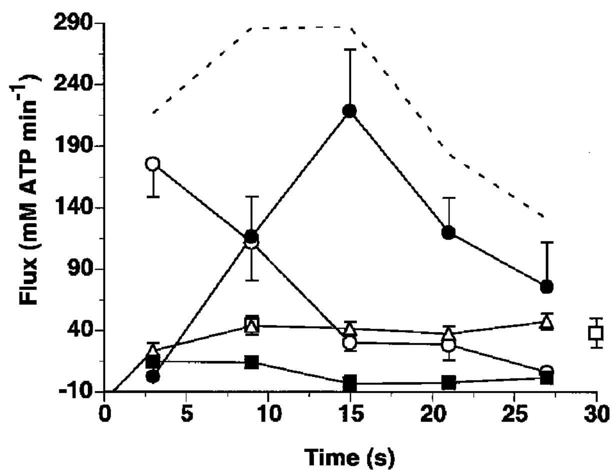

Figure 6. ATP provisions in the medial gastrocnemius during maximal rate exercise

ATP flux as supplied by creatine kinase (○), aerobic metabolism (□, measured; △, calculated), glycogenolysis (●) and net ATP breakdown (■). The dashed line represents total ATP flux. Data are means ± S.E.M.

at the onset of maximal-rate exercise, net ATP synthesis by creatine kinase (mM min^{-1}) accounted for the major part of the total ATPase flux (80%) and decreased throughout exercise to 5% of the total ATPase rate at the end of exercise. Due to the rapid fall in PCr and increased P_i levels, aerobic ATP synthesis reached 86 ± 1% of Q_{max} within 10 s, but only contributed 10–30% to the total ATPase flux. Total ATPase flux at the onset of high intensity exercise was 287 mM ATP min^{-1} and decreased by 46% at the end of 30 s. A peak glycogenolytic ATP synthesis rate of 219 ± 50 mM ATP min^{-1} was measured within 15 s (Fig. 6).

DISCUSSION

In this study we measured *in vivo* glycolytic rates, proton efflux rates (V_{eff}), proton consumption/production rates, and the apparent buffer capacity in a single muscle using a one-dimensional MRI-guided localization technique. Previous spatially localized measurements of anaerobic ATP synthesis rates have depended on surface coil localization. Using a 1D-localization procedure we were able to monitor intracellular pH and PCr kinetics in a single muscle during exercise and recovery, with a temporal resolution of 6 s. This permitted the non-invasive measurement of ATPase rates under exercise conditions known to elicit peak anaerobic ATP synthesis rates.

Buffer capacity

The apparent buffer capacity of the medial gastrocnemius was measured during step changes in ATPase flux. The total buffer capacity was calculated based on changes in pH_i in response to a change in proton load caused by the rapid hydrolysis of PCr at the onset of high intensity exercise. The non-phosphate, non-bicarbonate buffer capacity (β_i) was calculated based on changes in P_i and pH_i under the assumption that the cytosol was closed to CO_2 (Kemp *et al.* 1993). We found that at the onset of high intensity exercise the medial gastrocnemius had an apparent buffer capacity (β_{tot}) of $41·4 \pm 6·6$ slykes and a β_i of $27·0 \pm 6·2$ slykes at a pH of 7·14. This value is in accordance with previous unlocalized ^{31}P measures, which report β_i values of 24 ± 5 and 17·4–30·0 slykes in the triceps surae (Newcomer *et al.* 1999) and wrist flexor muscle groups (Kemp *et al.* 1993). Based on whole muscle homogenates, estimates of β_{tot} in humans range from 40 to 78 slykes (Hultman & Sahlin, 1980; Spriet *et al.* 1987). If allowances are made for changes in P_i, PCr and ATP, and glycolytic intermediate accumulation, β_i has been predicted to be approximately 31 slykes at a pH_i of 6·6 (Hultman & Sahlin, 1980). During exercise the major contributor to changes in β_{tot} is [P_i] (Hultman & Sahlin, 1980; Adams *et al.* 1990; Kemp *et al.* 1993). Therefore, β_{tot} cannot be considered to be static and will vary according to activity levels, pH_i and primarily the P_i content.

Proton and lactate efflux rates

The observed changes in pH_i and cytoplasmic lactate concentration were corrected for their buffering of H^+ and transport of lactate by the circulatory system. The dependence of PCr and pH_i recovery kinetics on proton efflux was calculated assuming that the lactate–proton transporter is the dominant mechanism for lactate and proton extrusion during exercise and recovery (Pilegaard *et al.* 1995; Juel, 1997). Proton efflux rates were calculated based on the reported linear dependence of lactate–H^+ efflux rates on the myocyte–interstitial fluid pH gradient (Bangsbo *et al.* 1993; Kemp *et al.* 1993; Richardson *et al.* 1998). Based on biexponential fits to both PCr and pH_i recoveries, the peak *in vivo* lactate–proton coefflux rate in the medial gastrocnemius was determined to be $15·3 \pm 2·7$ mM min^{-1}. This efflux rate is within the range of 5–22 mM lactate min^{-1} measured in the quadriceps following intense exercise (Bangsbo *et al.* 1993; Richardson *et al.* 1998). The pH_i recovery rate measured in the medial gastrocnemius ($0·30 \pm 0·04$ pH units min^{-1}) was faster than the rates previously reported in the human forearm ($0·15 \pm 0·02$ pH units min^{-1}) or the quadriceps (0·08 pH units min^{-1}). The experimentally observed linear dependence of the efflux rate on pH_i agrees with values previously measured in the triceps surae (Newcomer *et al.* 1999) and quadriceps muscle groups (Richardson *et al.* 1998).

The calculated *in vivo* proton efflux rate is an overestimate of the *in vivo* lactate efflux rate. Both MRS (Pan *et al.* 1991) and *in situ* measurements have reported differences in the recovery time courses of pH_i and muscle lactate (Bangsbo *et al.* 1993; Pilegaard *et al.* 1995; Juel, 1997). In this study we assumed that the only mechanism for the lactate and proton disposal is the 1:1 cotransport of lactate and protons; however, the removal of each ion also occurs via simple diffusion of undisassociated lactic acid and by internal metabolism (Juel, 1997). The rate of removal of lactate by either internal metabolism or diffusion of undisassociated lactate has been shown to be negligible (Pilegaard *et al.* 1995; Juel, 1997). It has also been put forth that protons are transported by Na^+–H^+ and HCO_3^-–Cl^- exchangers (Kemp *et al.* 1994; Juel, 1997). The ratio of proton-to-lactate efflux ranges between 1 and 1·5 in human muscle during recovery (Bangsbo *et al.* 1993; Juel, 1997). This ratio agrees with that predicted based on the individual activities of the lactate–proton cotransporter, and the Na^+–H^+ and HCO_3^-–Cl^- transporters measured in rat skeletal muscle (Juel, 1997). Furthermore, the lactate–proton transport capacity was found to be more than twofold greater than the sum of the other two exchangers (Juel, 1997). This ratio is not expected to remain constant throughout the recovery period due to different transport sensitivities to [lactate], pH_i and blood flow (Pilegaard *et al.* 1995; McKenna *et al.* 1996). Assuming a worst case scenario, that the *in vivo* lactate efflux rate is overestimated by 50%, the peak lactate accumulation rates during a 30 s maximal rate exercise would change by only 1%. Moreover, glycolytic flux (mM ATP min^{-1}) is calculated based on the buffering of protons generated by glycogenolysis and the amount of lactate retained does not affect these calculations.

The dependence of lactate efflux on the muscle interstitial lactate and proton gradients suggests that blood flow could play a prominent role in determining efflux rates. However, animal studies have shown that there is no hindrance to lactate efflux at blood flows greater than 0·6 ml min^{-1} g^{-1} (Pilegaard *et al.* 1995). In humans, blood flow is typically in the range 2·5–3·8 ml min^{-1} g^{-1} at the end of maximal exercise (Richardson *et al.* 1998). Since it is difficult to directly alter human blood flow rates at high lactate and proton levels, human studies investigating the effect of blood flow on lactate and H^+ efflux have relied on measurements during active (zero external resistance) and passive recovery from intense exercise. Bangsbo *et al.* (1993), using *in situ* measurements of the quadriceps, found that blood flow had no effect on lactate or proton efflux rates. On the other hand, Yoshida *et al.* (1996), using ^{31}P-MRS, reported a difference in pH_i recovery during active *versus* passive recovery. They found that the linear pH_i recovery increased from 0·014 ± 0·02 to 0·095 ± 0·02 pH units min^{-1} during active recovery in the quadriceps muscles. In comparison, we measured a pH_i recovery rate of 0·19 ± 0·03 pH units min^{-1} (0·06–0·36 pH units min^{-1}) in the medial gastrocnemius, indicating that pH_i recoveries following this exercise protocol are probably not blood flow limited.

Total ATP synthesis rates

At the onset of high intensity exercise, total ATPase rates are immense and are initially supported by high energy phosphate hydrolysis (Jones *et al.* 1985; Spriet *et al.* 1989; Greenhaff *et al.* 1994; Putman *et al.* 1995; Bogdanis *et al.* 1996; Casey *et al.* 1996; Trump *et al.* 1996). In the medial gastrocnemius, we measured a total ATP flux of 217 mM ATP min^{-1} at the onset of maximal rate exercise which was primarily supported by creatine kinase (80% of the total ATP synthesis). Aerobic ATP synthesis achieved 85 ± 2% of its maximal capacity within 9 s and did not change throughout the exercise. Moreover, aerobic ATP synthesis only represented 10–30% of the total ATP flux. This initial high dependence of ATP synthesis rates on creatine kinase is consistent with the recent observations of the lack of burst activity in creatine kinase deficient mice (van Deursen *et al.* 1993).

Within seconds of high intensity exercise, glycolysis was elevated to peak values. In this study glycolytic rates were found to peak at 146 ± 33 mM lactate min^{-1} within 15 s, decreasing to 51 ± 24 mM lactate min^{-1} at the end of 30 s. This transient increase in glycolysis during this exercise is similar to that observed *in situ* (Boobis *et al.* 1982; Jacobs *et al.* 1983; Jones *et al.* 1985; Greenhaff *et al.* 1994; Putman *et al.* 1995; Bogdanis *et al.* 1996; Casey *et al.* 1996). Several *in situ* studies have shown that within 10 s of maximal running or cycling, a peak lactate production rate of 110 mM lactate min^{-1} can be reached in the human quadriceps (Boobis *et al.* 1982; Jacobs *et al.* 1983; Jones *et al.* 1985). Further confirmation that glycolytic rates of this magnitude can be achieved *in vivo* is based on the lactate accumulation and glycogen degradation rates measured during direct muscle stimulation. Several studies have reported lactate accumulation rates of 80–100 mM lactate min^{-1} within 5 s of 50 Hz direct muscle stimulation with occluded circulation (Hultman & Sjöholm, 1983; Söderlund *et al.* 1992). Thus, glycolytic ATP synthesis rates as high as 219 ± 50 mM ATP min^{-1} can be achieved in the medial gastrocnemius during short duration, high intensity exercise. The ratio of peak glycolytic ATP synthesis rate to maximal oxidative ATP synthesis in this study was 2·94 ± 0·86. This ratio is consistent with the ratio of maximal *in vitro* activities of phosphofructokinase to oxoglutarate dehydrogenase (Blomstrand *et al.* 1986) in the vastus lateralis muscles of differently trained individuals. These results clearly demonstrate the immense 'burst' potential of human skeletal muscle and its enormous capacity to generate ATP anaerobically.

During high intensity exercise, glycogenolytic flux is greater than glycolytic flux. Based on the build-up of PMEs during exercise, we measured a peak glycogenolytic rate of 1·88 ± 0·43 glucosyl units s^{-1}, which is 35% greater than the peak glycolytic rate. The excessive build-up of PMEs during exercise is the hallmark of phosphofructokinase-deficient muscles (Duboc *et al.* 1987). But even in healthy subjects substantial PME accumulation can occur following high intensity exercise (Vandenborne *et al.* 1991). Based on *in situ* measurements, the major constituent of the PME peak during high intensity exercise is glucose 6-phosphate (Boobis *et al.* 1982; Hultman & Sjöholm, 1983; Jacobs *et al.* 1983; Jones *et al.* 1985; Greenhaff *et al.* 1994; Putman *et al.* 1995; Bogdanis *et al.* 1996; Casey *et al.* 1996). The *in situ* glucose 6-phosphate concentration in healthy muscles increases from 0·4 mM at rest to 9 mM during 30 s of high intensity exercise (Boobis *et al.* 1982; Jacobs *et al.* 1983; Jones *et al.* 1985; Greenhaff *et al.* 1994; Putman *et al.* 1995; Bogdanis *et al.* 1996; Casey *et al.* 1996). We measured a PME concentration of 11·9 ± 1·8 mM at the end of exercise following the correction for IMP accumulation, and attribute this build-up primarily to glucose 6-phosphate accumulation.

IMP formation when coupled to ATP hydrolysis and P_i release results in an uptake of protons (Hultman & Sahlin, 1980). In this study we measured a 34% decrease in the ATP level during the exercise, and this is in agreement with 17–34% ATP depletion rates measured *in situ* during high intensity exercises (Boobis *et al.* 1982; Jacobs *et al.* 1983; Jones *et al.* 1985; Greenhaff *et al.* 1994; Putman *et al.* 1995; Bogdanis *et al.* 1996; Casey *et al.* 1996). A 34% decrease in ATP, at a pH_i of 6·5, results in the net uptake of 0·8–1·6 mM protons (Hultman & Sahlin, 1980). Therefore, *in vivo* peak glycolytic rates in this study may be subject to a small error (±1%), incurred by the net uptake of protons via IMP formation.

The dependence of the glycolytic rate on inorganic phosphate was examined during high intensity exercise conditions. Glycogenolytic control in muscle is known to be under hormonal, substrate and allosteric regulation

permitting a rapid burst in glycolytic rate at the onset of muscle activity. Paradoxically, even in the presence of high concentrations of all the activators, glycolysis cannot occur without muscle contraction (Quistorff *et al.* 1992). Chasiotis (1996) reported that phosphorylase activity *in vivo* can be described by Michaelis–Menten kinetics with a K_m for P_i of 28 mM in the absence of AMP and 6·8 mM in its presence. Our findings support their observation that glycolytic rate is elevated in the presence of elevated [P_i], but not their conclusion that glycolysis follows Michaelis–Menten kinetics *in vivo* (Fig. 5). This result, in addition to the observed accumulation of hexosemonophosphates, indicates that phosphorylase is not the rate limiting enzyme during high intensity exercise, when glycogen levels are above 1–2 mM glucosyl units (Chasiotis, 1996). Moreover, this study shows that the glycolytic and glycogenolytic fluxes decrease while [ADP] and [AMP] remain elevated. The latter results support the notion that glycolysis behaves as a 'feed forward' system controlled by Ca^{2+}, independent of metabolite level (Conley *et al.* 1997).

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Acknowledgements

We would like to thank Kevin McCully for his support and insight. This work was supported by NIH grants RR02305 and R29-HD33738. The laboratory work was carried out at the Metabolic Magnetic Resonance Research & Computing Center, Department of Radiology, University of Pennsylvania, Philadelphia.

Corresponding author

G. Walter: MMRRCC, Department of Physiology, University of Pennsylvania, Basement Blockley Hall, Philadelphia, PA 19104-6021, USA.

Email: glenn@ginger.mmrrcc.upenn.edu