# Absence of phosphocreatine resynthesis in human calf muscle during ischaemic recovery

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Changes in the metabolites phosphocreatine (PCr), $P_i$ and ATP were quantified by $^{31}P$ n.m.r. spectroscopy in the human calf muscle during isometric contraction and recovery under ischaemic conditions. Time resolution of the measurements was 10 s. During a 30–60 s ischaemic isometric contraction, PCr decreased linearly at a rate of 1.17 %/s (relative to the resting value) at a contraction strength equivalent to 70 % of the maximal voluntary contraction (MVC) and at a rate of 2.43 %/s at 90 % MVC. There was a corresponding increase in $P_i$ but the concentration of ATP did not change. pH decreased linearly during contraction by 4.22 and 8.23 milli-pH units/s at 70 and 90 % MVC respectively. During a subsequent 5 min interval of ischaemic recovery, PCr, $P_i$, ATP, phosphomonoesters and calculated free ADP, free AMP and pH retained the value they had attained by the end of contraction with no significant recovery. Thus it is concluded that anaerobic glycolysis and glycogenolysis is halted momentarily on termination of contraction and that PCr is not resynthesized during ischaemic recovery. This paradoxical arrest of glycolytic flow in spite of the very significantly elevated concentration of potent activators such as $P_i$ and free AMP clearly indicates that parameters other than PCr, ATP, $P_i$, calculated pH, free ADP and free AMP regulate glycolysis and glycogenolysis of human skeletal muscle very efficiently under ischaemic conditions.

## INTRODUCTION

Recovery from exercise normally involves the restoration of several ionic and metabolic perturbations characteristic of the contracting muscle. Consequently, compared with the resting state, the process of recovery under aerobic conditions is coupled with an increased ATP turnover in the muscle, as demonstrated by the elevated post-exercise $O_2$ utilization. In contrast, under anaerobic conditions, post-exercise ATP turnover seems to be very low and phosphocreatine (PCr) appears to be 'frozen' at the low post-exercise level [1–4]. Furthermore, since there is no further fall in intracellular pH [3,4] or accumulation of lactate [5], the rapid glycolytic flux during exercise appears to be switched off. During the ischaemic post-exercise period, $P_i$ remains elevated corresponding to the lowered PCr content, and, assuming that the creatine kinase (CK) and adenylate kinase (AK) reactions are at equilibrium, very significant increases in the concentration of free ADP and free AMP may be calculated. The low glycolytic rate despite the presence of high concentrations of such potent activators ($P_i$ and AMP) of phosphofructokinase and glycogen phosphorylase suggests that these metabolites are not the main triggers of glycolysis and glycogenolysis during contraction [1,4]. For these reasons it was suggested that glycolysis is controlled by some other factor closely related to the contraction process, e.g. $Ca^{2+}$ [1]. However, the time resolution of PCr and pH were quite low in these measurements by $^{31}P$ n.m.r. (2 min; [1]) and with muscle-biopsy technique (0.5 min; [2]). Since no measurements could be made during the early recovery period, a partial resynthesis of PCr and lactate formation during the immediate post-exercise ischaemic period cannot be excluded. Partial resynthesis of PCr and lactate formation was in fact observed in anaerobic electrically stimulated frog muscle at 4 °C [4].

The purpose of the present study was to reinvestigate this phenomenon of paradoxical regulation of glycolytic flux in order to test whether partial resynthesis of PCr and a further fall in pH occurs in human muscle during the early ischaemic recovery period. We have used $^{31}P$ n.m.r. spectroscopy to measure PCr, $P_i$, ATP and pH in human muscle during anaerobic contraction and recovery with a time resolution of 10 s.

## MATERIALS AND METHODS

### Subjects

The experiments described were carried out on eight healthy male volunteers, participating in the study with informed consent. Mean age, height and weight for the subjects were 25.4 years, 176.4 cm and 67.8 kg respectively.

### Exercise protocol

The exercise involved isometric contraction of the calf muscle at 70 % and 90 % MVC under ischaemic conditions (Figure 1). MVC was determined as the average force measured for three consecutive maximal contractions, each lasting 3–5 s. Each experiment involved a 5 min rest period after the subject had been positioned in the magnet (see below). A cuff around the thigh was then inflated to a pressure of 32 kPa, ensuring ischaemic conditions of the calf muscle during contraction and recovery [6]. After an additional 5 min of rest, a voluntary isometric contraction of the calf muscle was initiated and the metabolic response was recorded on the n.m.r. instrument at a time resolution of 10 s. Target force was either 70 % or 90 % of MVC and the contraction was maintained for about 60 and 30 s respectively, causing a PCr decrease of 40–60 % in all experiments. The contraction was stopped so that the following spectrum (the first recovery spectrum) was recorded less than 1 s after the subject was given the command to stop the contraction. After about 300 s of ischaemic recovery, the cuff was released and the aerobic recovery was followed for 2–5 min.

### N.m.r. spectroscopy

The experiments were carried out in a Magnex magnet, 26 cm

Abbreviations used: PCr, phosphocreatine; MVC, maximal voluntary contraction; CK, creatine kinase; AK, adenylate kinase.

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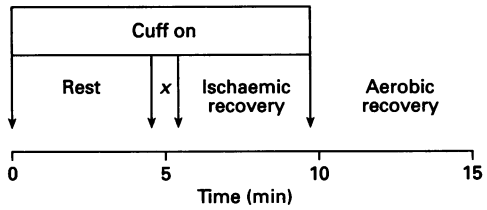


**Figure 1 Exercise and recovery during ischaemia in the human calf muscle**

Ischaemia was introduced by inflating a cuff around the thigh to 32 kPa [6]. $^{31}P$ n.m.r. spectroscopy was performed at a time resolution of 10 s throughout the experiment. Isometric contraction of the calf muscle was performed in the n.m.r. instrument with a pedal which allowed the force to be recorded [7]. $x = 50$–80 s for 70% MVC and 25–40 s for 90% MVC.

bore diameter and 80 cm bore length, interfaced to an Otsuka VivoSpec spectrometer. The leg was positioned in the magnet against a pedal [7] which allowed the applied force to be recorded by a calibrated strain gauge while the subject was sitting in a chair rigidly fixed to the ergometer pedal and the magnet. The RF coil was a 35 mm-diameter two-turn inductively driven surface coil on which the medial part of the gastrocnemius muscle was placed approximately 12 cm distal of the fossa poplitea. The magnet was pre-shimmed on the proton signal from muscle water and subsequently fine-shimmed on the PCr signal. A linewidth of the PCr resonance better than 20 Hz at rest was obtained in all experiments. Spectrometer frequency for phosphorus was 49.85 MHz. Spectrometer conditions were as follows: pulse width 60 $\mu$s, corresponding to a 180° pulse for a 1 ml spherical sample in the coil centre. Interpulse delay was 5 s. These recording conditions give some signal saturation (see below). Spectral width was 10 KHz and data were collected in 2 K data points, zerofilled to 4 K before Fourier transformation. Signal to noise ratio for two-scan spectra (10 s) was typically 12–15 (see Figure 2). Processing of the data involved integrations of the peak area using the Otsuka spectrometer software and correction for partial saturation (see below). Intracellular pH was calculated from the difference in chemical shift between $P_i$ and PCr as previously described [3]. Free ADP was calculated assuming CK equilibrium, and free AMP was calculated assuming AK equilibrium, with equilibrium constants of $1.66 \times 10^9\ M^{-1}$ and $1.12 \times$ respectively [8]. For details see the legend to Table 4.

The saturation factors of PCr, $P_i$ and the three ATP peaks were calculated by comparing the data obtained under the conditions described above with data obtained with an interpulse delay of 25 s. This comparison was carried out at rest, and, in order to test whether the saturation factors were affected by the contraction-induced changes, the experiment was also carried out at three different levels of contraction intensity characterized by $P_i$/PCr ratios [9] of approximately 0.5, 1.0 and 2.0. These levels of $P_i$/PCr ratio were obtained by appropriately timed isometric contraction of the ischaemic leg before the saturation measurement. Subsequently the two sets of data (eight scans of 5 s and eight scans of 25 s interpulse delay) were collected in an interleaved fashion in order to ensure full comparability. The $P_i$/PCr ratio deviated less than 10% from the end-contraction level during the 6 min recording interval for the individual saturation experiment. The specific saturation factors observed (see Table 3) were applied in the quantitative analysis of the data.

## RESULTS

The particular aim of this series of experiments was to provide a temporal analysis with a high time resolution of contraction and recovery during ischaemia. Figures 2(a) and 2(b) show stack plots of typical experiments at 10 s time resolution carried out at 70% and 90% MVC according to the protocol explained in detail in Figure 1. The spectra are displayed with a line-broadening of 10 Hz with arrows indicating the events of the exercise protocol. Figures 3(a) and 3(b) show the time course in one typical subject for the changes in pH and peak area of PCr, $P_i$ and ($P_i$+PCr) for experiments at 70% and 90% MVC respectively. During the 5 min interval of resting ischaemia, there was no significant change in the spectrum. The subsequent ischaemic contraction, however, caused a rapid apparently linear decrease in PCr and a corresponding linear increase in $P_i$ (Figures 2 and 3). The slopes of these changes have been calculated for 70% and 90% MVC, and are given in Table 1, expressed as percentage change/s relative to the individual PCr value at rest. At 70% MVC, the rate of decrease in PCr amounts to approx. 1%/s with a similar value for the $P_i$ increase. At 90% MVC the average rate of PCr decrease had more than doubled to 2.4%/s whereas the rate of $P_i$ increase only increased to 1.8%/s, which

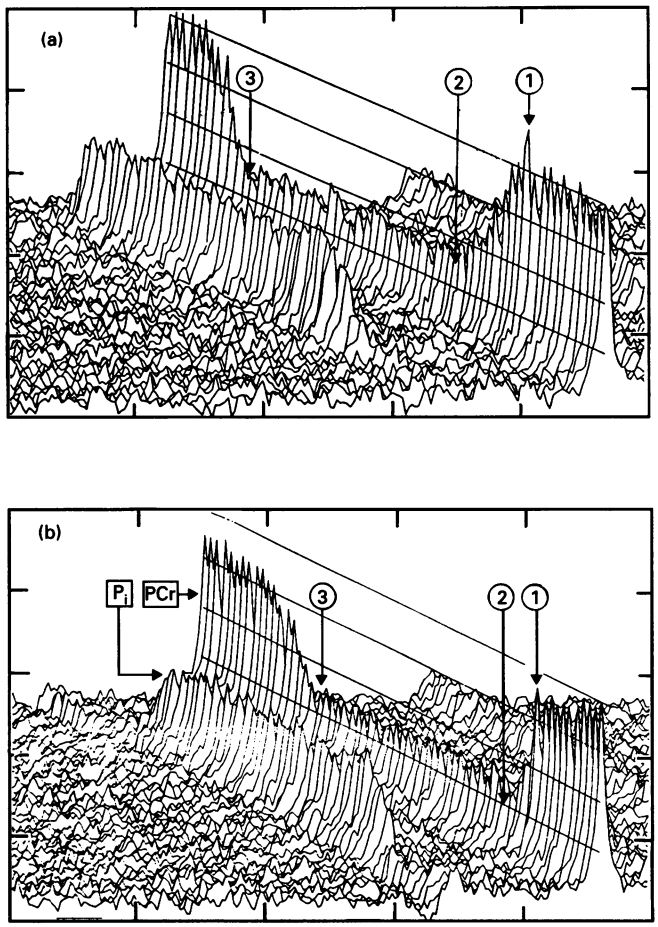


**Figure 2 Stacked $^{31}P$ n.m.r. spectra obtained from human calf muscle during ischaemic contraction and recovery**

(**a**) Typical experiment at 70% MVC; (**b**) typical experiment at 90% MVC. The subjects were positioned with one leg in the magnet, resting the foot on an ergometer pedal allowing force registration during isometric contraction. The exercise protocol is explained in Figure 1 and involved ischaemia at rest, contraction for the interval between ① and ②, ischaemic recovery between ② and ③, followed by aerobic recovery after the cuff release at ③. Each spectrum in the stack represents two scans, displayed with a line-broadening of 10 Hz. The horizontal lines of the stack plot indicate the 100%, 75%, 50% and 25% level relative to the resting value of the PCr peak. Only the part of the spectrum containing PCr, $P_i$ and phosphomonoester are included.

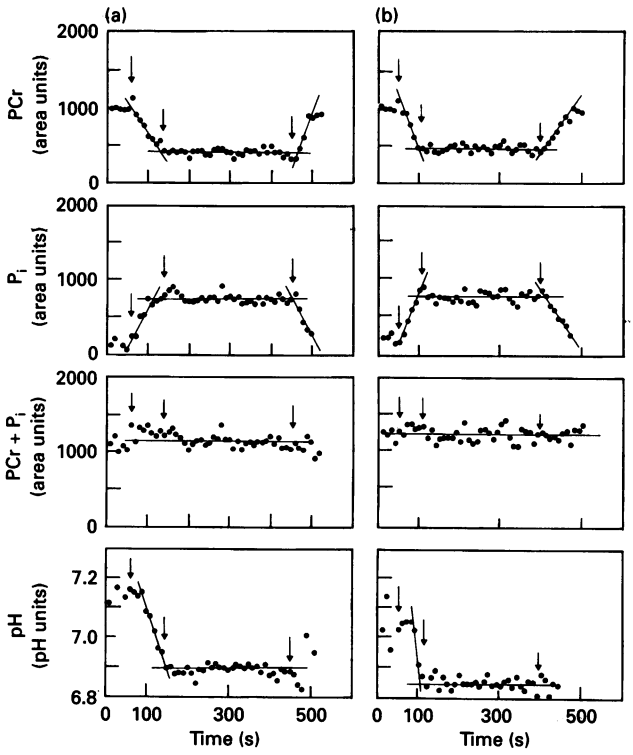


**Figure 3 Effect on PCr, $P_i$ and pH of isometric contraction and recovery during ischaemia followed by aerobic recovery**

(**a**) Typical experiment at 70% MVC; (**b**) typical experiment at 90% MVC. The conditions were as explained in the legend to Figure 2. Linear changes were assumed and the slopes indicated on the graphs were calculated for the individual experiments applying a least-squares method of linear regression. These data are given in Table 1.

was significantly different from the PCr value (Table 1). The pH did not change during the initial 5 min of ischaemia at rest, as calculated from the chemical shift difference between $P_i$ and PCr. However, during contraction there was a linear decrease of 4.22 milli-pH units (munits)/s for 70% MVC, and at 90% MVC the rate of decrease almost doubled to 8.23 munits/s (Table 1).

When contraction was terminated, the first scan was recorded within 1 s, and the second scan after an additional 5 s. Thus, since each spectrum is the sum of two scans, the first time point of recovery corresponds to 3.0–3.5 s after the command to terminate the contraction. The second time point corresponds to 13–13.5 s and so on. At this time resolution, it is apparent that the level of PCr, $P_i$ and pH is maintained unchanged during the 5 min of ischaemic recovery, i.e. slopes of change for these parameters were not significantly different from zero (Table 1 and Figures 2 and 3).

After 5 min of ischaemic recovery, the cuff was released and aerobic recovery was followed for 2–5 min. Again, the n.m.r. recording was synchronized so that the first scan reflects the time point 3–3.5 s after the release of the cuff. Recovery starts immediately after release of the cuff, as demonstrated in Figures 2 and 3. Assuming a linear rate of recovery for the first 40–50 s of the aerobic recovery (Figures 2 and 3), the rates of PCr and $P_i$ recovery were calculated as shown in Table 1, expressed as percentage change per s relative to the individual PCr value at rest. About 1%/s of recovery was found for both PCr and $P_i$ with no difference between 70% and 90% MVC. Assuming a biexponential recovery as described by Harris et al. [2] and calculating the initial rates of change from the data fitted to such a function, the same value of about 1%/s was found. However, owing to the insufficient memory capacity of our system, the time of observation of aerobic recovery was too short for precise kinetic analysis of the aerobic recovery process. Because of the transient decrease of the $P_i$ signal below the resting level, as was frequently observed during recovery (e.g. [10]), reliable pH calculation in our two-scan spectra was not feasible in most cases during aerobic recovery and these data are therefore not included in Figure 3. There was no significant change in ATP concentration in any of the experiments.

In supplementary experiments with three subjects, a second contraction was performed after 2–3 min of ischaemic recovery achieving the same target force as during the first contraction (i.e. 70% MVC) but lasting only 20–30 s. The rate of the changes during this second contraction was comparable with the first and again, on termination of the contraction, the $^{31}P$ n.m.r. spectrum remained constant in spite of the very significant decrease in PCr and increase in $P_i$ (Figure 4) [4].

### Stability of metabolite concentrations during prolonged ischaemia

The experiments described above covered intervals of ischaemia of approx. 5 min at rest and ischaemic recovery after contraction. From these data, it was concluded that the slope of PCr, $P_i$ or pH versus time was not significantly different from 0 (Table 1). In

**Table 1 Rate of change in PCr and $P_i$ concentration and pH in the calf muscle during ischaemic contraction, anaerobic recovery and aerobic recovery**

The experiments followed the protocol explained in Figure 1. The change in the concentrations of PCr and $P_i$ is given as %/s relative to the PCr value at rest of the individual experiment. The changes in [PCr], [$P_i$] and pH were assumed to be linear (cf. Figure 3) and were calculated applying a least-squares method. Results are given as means ± SD. *70% MVC vs. 90% MVC; $P < 0.05$. † |PCr| vs. |$P_i$|; $P < 0.05$.

| | Rate of change (%/s) | | Rate of change in pH (munits/s) |
|---|---|---|---|
| | PCr | $P_i$ | |
| 70% MVC ($n = 7$) | | | |
| Ischaemic contraction | −1.17 ± 0.26* | 1.02 ± 0.32* | −4.22 ± 0.71* |
| Ischaemic recovery | −0.019 ± 0.0090 | −0.016 ± 0.028 | −0.026 ± 0.038 |
| Aerobic recovery | 1.24 ± 0.41 | −1.28 ± 0.40 | – |
| 90% MVC ($n = 6$) | | | |
| Ischaemic contraction | −2.43 ± 0.38 | 1.77 ± 0.50† | −8.23 ± 2.06 |
| Ischaemic recovery | −0.002 ± 0.007 | 0.004 ± 0.033 | −0.09 ± 0.06 |
| Aerobic recovery | 1.12 ± 0.51 | −0.78 ± 0.12 | – |

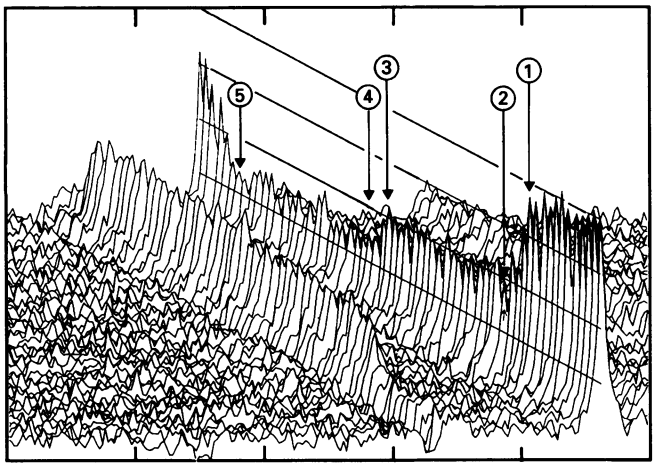


**Figure 4 Stacked $^{31}P$ n.m.r. spectra of the human calf muscle during ischaemic contraction and recovery**

The exercise protocol was as explained in the legend to Figure 1, except that in this experiment there were two contraction intervals, between ① and ② and between ③ and ④. Aerobic recovery started at ⑤.

**Table 2 Changes in [PCr] and pH during extended ischaemia at rest and during recovery**

Ischaemia of the human calf muscle was introduced by a cuff around the thigh inflated to 32 kPa. Subsequently the $^{31}P$ n.m.r. spectrum was followed for 20 min at rest. In separate experiments, an isometric contraction at 70% MVC was performed for 30–40 s in order to cause a 40–60% decrease in PCr. Subsequently the $^{31}P$ n.m.r. spectrum was followed for 13–15 min. The change observed in the PCr peak area and pH was linear with time, as evaluated by a least-squares linear regression method and all slopes were significantly different from zero. The PCr data are given as %/min relative to the preischaemic value, and the data for pH are given as munits/min. Values represent means ± S.D. of three experiments. *Significantly different from rest value; $P < 0.05$.

| | Rates of change | |
|---|---|---|
| | PCr (%/min) | pH (munits/min) |
| Ischaemic rest | −1.89 ± 0.24 | 4.90 ± 1.0 |
| Ischaemic recovery | −1.42 ± 0.18* | −3.14 ± 1.56* |

order to get a more accurate determination, we studied the time course of PCr, $P_i$ and pH during an extended ischaemic interval at rest or ischaemic recovery of approx. 20 and 15 min respectively in three subjects. These results, which are summarized in Table 2, show that at rest there was indeed a slight, but significant, decrease in PCr of 1.89%/min, whereas during ischaemic recovery after contraction for 30–40 s at 70% MVC, resulting in a decrease in PCr of 40–60%, the slope was 1.42%/min. This was lower than at rest but still significantly different from 0. During the extended period of ischaemia at rest, the pH increased slightly at a rate of 4.9 munits/min, but decreased by 3.14 munits/min during the 13 min ischaemic interval after an isometric contraction. Both slopes were significantly different from 0.

### Saturation factors

Owing to the difficulties involved in performing $T_1$ (spin-lattice relaxation time) measurements *in vivo* with a surface coil, it is common practice to evaluate n.m.r. signal saturation by comparing the spectra recorded with the applied interpulse delay with spectra accumulated under identical conditions except that the interpulse delay is increased severalfold. In the present experiments, we have compared 5 and 25 s interpulse delay as shown in Table 3. Since contraction causes a very significant change in the ionic composition of the intracellular space, it is possible that saturation factors might change between resting conditions and contraction. However, to our knowledge this has not previously been tested. In the present study we have evaluated such possible contraction effects on spin-relaxation by 'clamping' the metabolic state at three different levels of exercise corresponding to a $P_i$/PCr ratio [9] of approx. 0.5, 1.0 and 2.0. As shown in Table 3, saturation factors were of the order of 0.85 with no significant difference between the different metabolic conditions. Average saturation factors of 0.80, 0.75, 0.85, 0.89 and 0.82 were observed for $P_i$, PCr, $\gamma$-ATP, $\alpha$-ATP and $\beta$-ATP, respectively.

## DISCUSSION

The main finding of the present study is that PCr cannot be resynthesized during anaerobic conditions in human skeletal muscle and that pH change, i.e. lactate formation, is intimately linked to the contraction process. By using $^{31}P$ n.m.r. with a high time resolution we could show that the value of PCr and pH

**Table 3 Saturation factors for some resonances of the $^{31}P$ n.m.r. spectrum of human calf muscle at rest and during metabolic perturbations characteristic of contraction**

Saturation factors were obtained by comparing peak areas of spectra (16 scans) recorded interleaved with 5 and 25 s interpulse delay on the ischaemic leg. The comparison was carried out at rest ($P_i$/PCr ≈ 0.1) and at three different levels of metabolic perturbation, i.e. a $P_i$/PCr ratio of approx. 0.5, 1.0 and 2.0. These levels of $P_i$/PCr ratio were obtained by appropriately timed isometric contractions of the ischaemic leg before the saturation measurement. Values represent means ± S.D. of three experiments.

| | | Peak area (5 s/25 s interpulse delay) | | | |
|---|---|---|---|---|---|
| Peak identity | Approximate $P_i$/PCr ratio during measurement... | 0.1 (Rest) | 0.5 | 1.0 | 2.0 |
| $P_i$ | | 0.71 ± 0.20 | 0.90 ± 0.15 | 0.80 ± 0.08 | 0.77 ± 0.10 |
| PCr | | 0.72 ± 0.02 | 0.73 ± 0.02 | 0.77 ± 0.05 | 0.79 ± 0.07 |
| $\gamma$-ATP | | 0.91 ± 0.08 | 0.79 ± 0.03 | 0.84 ± 0.11 | 0.86 ± 0.12 |
| $\alpha$-ATP | | 0.88 ± 0.05 | 0.88 ± 0.06 | 0.93 ± 0.03 | 0.86 ± 0.12 |
| $\beta$-ATP | | 0.86 ± 0.02 | 0.78 ± 0.08 | 0.78 ± 0.06 | 0.85 ± 0.60 |

**Table 4 Calculated values for ADP and AMP at rest at the end of contraction and during ischaemic recovery**

Free ADP and AMP was calculated making the following assumptions: (1) CK and AK are at equilibrium with equilibrium constants of $1.66 \times 10^9$ and 1.12 $M^{-1}$ respectively [8]. (2) A Cr/PCr ratio at rest of 0.50 [30,31] and a PCr concentration at rest of 18.5 mmol/kg wet weight [32]. (3) A constant sum of PCr and Cr [31] and 100% n.m.r. visibility of PCr and ATP [32]. Peak area, appropriately corrected for partial saturation (see the Materials and methods section), was applied to the calculation. Each peak area value is subsequently converted into mol/kg wet weight multiplying by the ratio: $(18.5 \times 10^{-3})/PCr_R$, where $PCr_R$ is the peak area of PCr at rest of the individual experiment. (4) The $H^+$ concentration was calculated as follows(1):

$$[H^+] = \text{antilog} - \{6.75 + \log[(\delta - 3.27)/(5.69 - \delta)\},$$

where $\delta$ is the chemical shift difference between the $P_i$ and PCr peaks. Thus

$$\text{Free [ADP]} = \frac{\text{ATP}\,[PCr_R\,0.50 + (PCr_R - PCr)]}{PCr \times H^+ \times 1.66 \times 10^9}$$

and

$$[AMP] = \frac{ADP^2 \times 1.12}{ATP}$$

Results are expressed as $\mu$mol/kg wet weight. Means ± S.D. are given. All values reflecting contraction and recovery are significantly different with respect to the rest value, $P < 0.01$.

| | Rest | | Contraction (last 10 s) | | Ischaemic recovery | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | First 10 s | | After 300 s | |
| | ADP | AMP | ADP | AMP | ADP | AMP | ADP | AMP |
| 70% MVC ($n = 7$) | 18.6 ± 3.9 | 0.13 ± 0.06 | 67.5 ± 24 | 1.03 ± 0.58 | 57.9 ± 20 | 0.88 ± 0.43 | 67.7 ± 21 | 1.06 ± 0.55 |
| 90% MVC ($n = 6$) | 14.2 ± 3.8 | 0.082 ± 0.03 | 49.4 ± 8.6 | 0.69 ± 0.21 | 50.7 ± 10.7 | 0.76 ± 0.46 | 47.3 ± 11.8 | 0.63 ± 0.12 |

observed after 3.5 s of ischaemic recovery remained unchanged after an additional 300 s of ischaemic recovery. The present data confirm previous findings obtained with a lower time resolution in studies of human muscle with $^{31}P$ n.m.r. technique [3] and with biopsy technique [2,11] or in studies of isolated rat muscle [5]. The partial resynthesis of PCr observed in anaerobic isolated frog muscle [4] may possibly be related to incomplete anaerobiosis or to altered enzyme kinetics due to the low temperature (4 °C).

The central aspect of the present results is the regulation of glycolysis. Normally, it is assumed that AMP and $P_i$ play major roles in the regulation of the rate of glycolysis (see [12] and references therein). In the present experiments, however, it is obvious that, although the rate of glycolysis during the ischaemic contraction is high as indicated by the rapid decrease in pH, it stops momentarily when contractions stops (Figures 2 and 3 and Table 1). By using the CK and AK equilibria for the calculation of free ADP and free AMP, it is furthermore apparent that both of these parameters are significantly elevated, AMP by about 10-fold (Table 4). Hence, in spite of the fact that the muscle content of PCr, $P_i$ and calculated free ADP and AMP remain unchanged at the level characteristic of contraction, glycolysis is stopped momentarily when contraction stops. Since there is also no sign of further glucose 6-phosphate accumulation (no increase in the phosphomonoester peak) during the ischaemic recovery after the termination of contraction (see Figure 2), it follows that glycogen phosphorylase must be regulated in concert with glycolysis. These results are consistent with previous work with a similar exercise protocol of ischaemic contraction and recovery in frog muscle [4], rat muscle [5] and human muscle [2,3], and it can be concluded that there must be factors other than $P_i$, PCr, ATP, pH, calculated free ADP and calculated free AMP that efficiently control both glycolysis and glycogenolysis of human skeletal muscle.

These factors must be intimately linked to the contraction process allowing very rapid switching from 'on' to 'off'. Cytosolic [$Ca^{2+}$] changes rapidly in concert with the contraction and has been suggested to be the controlling parameter [1]. It is well documented that an increase in cytosolic [$Ca^{2+}$] can activate glycogenolysis through transformation of phosphorylase from the *b* to the more active *a* form. However, it has been shown in human muscle that glycogenolysis can be switched off after contraction despite the fact that almost all of the enzyme prevails in the active *a* form [13,14]. If therefore a decrease in $Ca^{2+}$ is the regulator of glycogenolysis during the rapid on/off transition when contraction is terminated, it must occur through some other mechanism than transformation of phosphorylase from the *a* to the *b* form. An increase in $Ca^{2+}$ has in some studies been shown to activate phosphofructokinase [15] and AMP deaminase [16] but the effects were small or absent during physiological conditions and it is doubtful if these effects are of physiological importance.

Another possible explanation of the observed coupling between the contraction process and glycogenolysis/glycolysis could be direct conformation changes in the relevant regulatory enzymes, possibly induced by the contraction process proper, which in turn would control the glycogenolytic and glycolytic flux. There are several reports describing a direct association between glycolytic enzymes and the contracting elements ([17–22]; for a review see ref. [23]). However, the exact functional nature of such a conformational coupling of glycolytic and glycogenolytic enzyme activity to contraction remains an elusive, albeit very attractive, idea.

The value of free ADP and free AMP have been calculated (Table 4) based on the assumption that the CK and AK reactions are close to equilibrium [8,24]. The validity of this assumption can be questioned during conditions of rapid decrease in PCr since an increase in ADP above that at equilibrium is a prerequisite to drive the CK reaction, and similarly for the AK reaction. Furthermore, the free ADP concentration appears to be far below the $K_m$ values for ADP of CK (800 $\mu$M [25] and AK (1600 $\mu$M [26]). It is therefore possible that the CK and AK reactions are out of equilibrium in contracting muscle, despite the high maximal enzyme activity. Studies using the n.m.r. saturation transfer technique have provided evidence that this may indeed be the case in contracting frog muscle [27]. In this work it was calculated that the breakdown of PCr during the first

3 s of contraction is driven by a large increase in the concentration of free ADP, more than 2-fold higher than the calculated equilibrium concentration of free ADP. In the transgenic mouse which expresses CK in the liver at comparable activities to that in muscle, Brosnan et al. [28] showed that a 5-fold change in CK activity was without effect on the calculated free ADP concentrations of 53 $\mu$M, attesting to CK equilibrium under those conditions. However, ATP turnover in liver is much lower than in contracting muscle.

All in all, it is important to realize that, although equilibrium of CK during contraction may be questioned for the reasons mentioned above, the fact that PCr remains almost unchanged during the 5 min of ischaemic recovery (see Figures 2 and 3) almost certainly indicates that equilibrium was attained at the time of the first recovery measurement, i.e. 3 s after the termination of contraction. This conclusion would seem to be wrong only if CK was completely inhibited during ischaemic recovery, and, although it has been reported that a combination of low pH and high $P_i$ might cause a pronounced inactivation *in vitro*. [29], the fact that PCr increases at a high rate immediately upon release of the cuff seems to exclude such a possibility *in vivo*. Thus there can be little doubt that both ADP and AMP concentrations remain high (see Table 4) until aerobic conditions are re-established on release of the cuff. Further analysis of the kinetic characteristics of CK and AK *in vivo* seem desirable in order to establish the significance of ADP and AMP as metabolic regulators in a contracting muscle.

## Conclusion

In conclusion, the present investigation demonstrates an extremely tight coupling in the normal human calf muscle between contraction and glycogenolysis/glycolysis, with no sign of metabolic recovery when ischaemic conditions are maintained after termination of contraction. Thus glycogenolysis and glycolysis are controlled in concert by some factor associated with the contraction proper, remaining almost completely halted, in spite of the presence of strongly stimulatory concentrations of the normal metabolic activators ADP, $P_i$ and AMP. The elucidation of the control of these central metabolic pathways in contracting muscle is an important challenge of biochemistry.

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