Energy Metabolism in Post Mortem Muscle

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INTRODUCTION

A dominant feature of biochemical events in post mortem muscle is the fall in pH. The rate and extent of the post mortem pH fall has im effects on meat quality, particularly when considered relative to the imposed chilling rate; the PSE condition in pork or cold shortening obvious consequences of, respectively, extremely fast and slow pH fall relative to temperature decline. The use of electrical stimulation become an important processing tool for accelerating pH fall independent of temperature changes. There are therefore important com justifications for understanding and manipulating the mechanisms controlling energy metabolism in the post mortem muscle.

ATP changes in post mortem muscle

In the resting muscle, Bendall (1973) has proposed that the rate of ATP hydrolysis is primarily determined by myosin ATPase activity grounds that the temperature dependence of post mortem pH decline is similar to the temperature dependence of myosin ATPase. In co resting muscle, electrical stimulation increases intracellular calcium ($[Ca^{2+}]_i$) and activates actomyosin ATPase to accelerate ATP turn more than 100 fold. However, as much as 40% of the ATP use under these conditions can be attributed to non-contractile processes, p Na^+-K^+-ATPase and Ca^{2+}-ATPase (Baker *et al.*, 1994).

Two separate mechanisms exist to regenerate ATP under the anaerobic condition of post mortem muscle, with differing effects on pH. The kinase reaction catalyses the resynthesis of ATP from ADP and creatine phosphate (CP), one consequence of which is proton consump

$$ATP + H_20 \rightarrow ADP + P_i + cH^+$$
$$PCr + ADP + H^+ \leftrightarrow creatine + ATP$$

$$PCr + H_20 + \alpha_pH^+ \rightarrow creatine + P_i$$

where α_a defines the proportion of produced H^+ that is unbound, as defined by muscle buffering, and where $\alpha_p = 1-\alpha_a$.

In contrast, ATP hydrolysis combined with resynthesis by glycolysis results in a net proton accumulation

$$3(ATP + H_20 \rightarrow ADP + P_i + \alpha_aH^+)$$
$$(Glucose)_n + 3ADP + 3P_i + \alpha_aH^+ \rightarrow (glucose)_{n-1} + 3ATP + 2lactate + 2H_20$$

$$(Glucose)_n + 3ADP \rightarrow (glucose)_{n-1} + 3ATP + 2lactate + 2\,\alpha_aH^+ + H_20$$

The majority of ATP resynthesis is therefore shared between two separate pathways. The pH decline in muscle is the sum of their respective on $[H^+]$, together with changes in muscle buffering produced by the chemical state of the phosphate groups.

Electrical stimulation

Electrical stimulation is used to accelerate the rate of pH decline in post mortem muscle. Its effects on pH have two distinct components (C & Devine, 1978). First, there is the decline produced directly by the muscular contractions, from the accelerated energy expenditure ass with the high levels of work done by the muscle. Second, subsequent to the electrical stimulation, the rate of pH fall is increased, so that, a the rate following stimulation is 50-70% greater than unstimulated control muscle. There remains therefore a sustained increase in the glycolysis, and therefore of ATPase activity, after the muscle returns to the resting state following stimulation, but the causes of this i remain to be fully described.

^{32}P-NMR measurements of post mortem energy metabolism

^{32}P-NMR is an effective technique for monitoring the energy metabolism in muscle (Meyer *et al.*, 1982; Vogel *et al.*, 1985; Renou *et al.* it allows a continuous and non-invasive measurement of changes in the concentrations of high energy phosphate compounds (ATP, CP, in phosphate (P_i) and hexose phosphate), and this information can be used to assess post mortem energy metabolism. In the experiments de here, strips of lamb m. semitendinosus were prepared with minimal damage to the fibres, and recordings made from the distal portion muscle. The muscles were mounted onto stainless steel wire guides and inserted into 10 mm diameter recording tubes. Anaerobic condition maintained by immersion in mineral oil. ^{32}P-NMR recordings were made using a Bruker AC300, with a multinuclear probe tuned for ^{32}P at MHz, and a 60° tip angle with 1 second relaxation delay. The peaks were quantified to μM/g wet weight according to the method of V *al* (1985). pH changes in the muscle were calculated from the shift of the inorganic phosphate peak. Unless stated otherwise, all recordings carried out at a constant temperature of 35°C.

The muscle samples were stimulated within the recording tubes using the wire guides as electrodes. A constant current stimulator was used, set to 50 mA, and a waveform of 10 ms pulses of alternating polarity at 15 Hz . The duration of stimulation was 60 seconds.

Post mortem energy metabolism in muscle held at 35°C.

Figure 1 shows the changes in the concentrations of phosphate compounds from a representative muscle. It follows the general pattern described in other species using biochemical determinations (Bendall, 1979) and ^{32}P-NMR (Vogel *et al.*, 1985; Renou *et al.*, 1986; Azuma *et al.*, 1994). A consistent difference was the absence of a delay phase (when ATP concentrations remain essentially constant; Bendall, 1973), as reported in previous studies. However, the delay phase was evident when the same muscle is held at 15°C, suggesting that the faster rate of ATPase activity at 35°C is responsible for the early onset of the decay phase.

Lactate accumulation can be used to estimate the rate of glycolysis, but cannot be measured directly from ^{32}P-NMR. However, a close and linear relationship has been recognised between lactate concentration in muscle and pH (Bendall, 1973; Fabiansson & Reutersward), a relationship that in lamb is unaffected by electrical stimulation or by temperature (unpublished observation), and offers therefore a simple conversion using the measured value of 58 μM lactate/pH/g wet weight. An alternative approach is to calculate the rate of glycolysis from changes in [H^+], if the buffering of muscle is accounted for. By titration of homogenised post rigor muscle to measure buffering, and calculating the effects of phosphate metabolism (hydrolysis of CP and ATP) on the total buffering, the calculated rate of glycolysis was equivalent to the much simpler pH-lactate relationship.

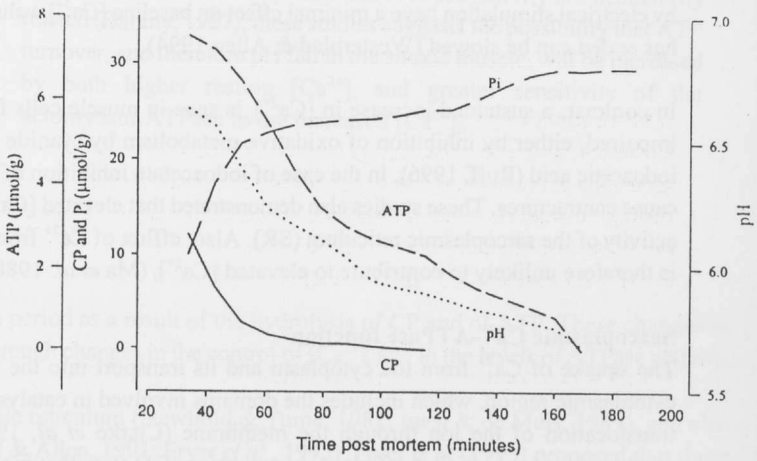

Figure 1. ^{32}P-NMR results from post mortem muscle.

The rate of ATPase activity during the pre rigor period can then be described from the NMR data. The rate of resynthesis of ATP (μM/g wet weight/minute) can be calculated from the stoichiometry:

$$\Delta ATP = \Delta PC + \Delta(1.5\ lactate) + \Delta(2ATP)$$

Figure 2 shows the rate of ATP turnover from a representative muscle during the pre-rigor period, expressed relative to pH rather than time post mortem. The rate of ATP turnover decreases progressively as the pH falls to pH 6.3, becomes stable until pH around 6.1 and then declines thereafter until rigor is complete. This result contrasts somewhat with those described by Bendall (1973), who noted a resurgence in ATP turnover in the intermediate pH range. The difference is probably associated with the earlier fall in ATP levels in lamb muscle at 35°C (the absence of a delay phase), since the resurgent pattern was evident in muscle analysed at 15°C (data not shown). Because ATP resynthesis can occur through either the creatine kinase reaction or by glycolysis, the proportional contribution of glycolysis to the total resynthesis during the pre rigor period is also shown in Figure 2. In the early post mortem period, when CP levels are still high, glycolysis contributes around 65% of the ATP resynthesis, but gradually rises to reach more than 90% once creatine phospahte is depleted. The remainder of the ATP activity is derived from the myokinase reaction.

Figure 3 shows the calculated rate of ATP turnover from a representative muscle following electrical stimulation. Overall, the rate of ATP turnover is increased by around 55% compared with unstimulated. As in unstimulated muscle, the rate of ATP turnover declines as the pH falls, but the decline is now much more pronounced. In contrast to unstimulated muscle, the rate of ATP turnover increases from pH 5.9 until rigor.

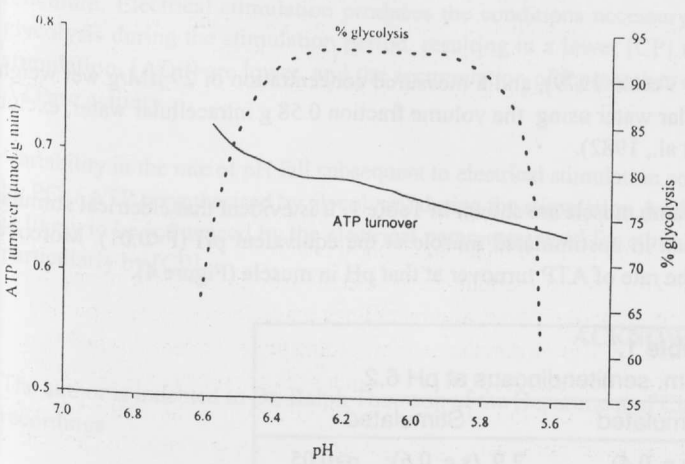

Figure 2. ATP turnover and % glycolysis in unstimulated muscle

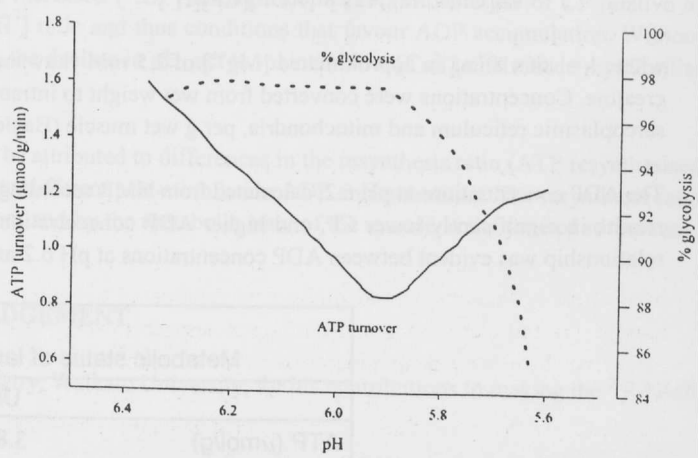

Figure 3. ATP turnover and % glycolysis in stimulated muscle

At an equivalent pH, CP levels are lower in stimulated muscle than in unstimulated muscle (Table 1). This means that the resynthesis rat proportion of resynthesised ATP derived from the creatine kinase reaction versus glycolysis, is increased during electrical stimulation. results showing high levels of PCr contribution to total ATP resynthesis in the early phases of an electrical stimulation protocol have been r in both anaerobic and aerobic muscle (Spriet *et al.*, 1987; Krause & Wegener, 1996)

Why is the rate of glycolysis accelerated in muscle following electrical stimulation?

While the pH fall during stimulation can be anticipated from the intense muscular activity, the cause of the accelerated rate of pH fall subs to the stimulation has not been effectively explained. The increased ATPase activity probably implicates elevated $[Ca^{2+}]_i$. Using Ca^{2+}-se fluorescent probe introduced into oxygenated fibres, the measured resting levels of $[Ca^{2+}]_i$ increase slightly when the muscle pH falls Ashley, 1978; Westerblad & Allen, 1993), for example from 26 to 40 nM in single mouse muscle as the pH falls from 7.3 to 6.8 (Weste Allen, 1993), but such changes in concentration are probably too small to generate significant metabolic effects. More substantial pH falls by electrical stimulation have a minimal effect on baseline $[Ca^{2+}]_i$ values, although the rate of recovery of $[Ca^{2+}]$ to baseline values once stim has ended can be slowed (Westerblad & Allen 1994).

In contrast, a sustained increase in $[Ca^{2+}]_i$ is seen in muscle cells following electrical stimulation under conditions where ATP resynt impaired, either by inhibition of oxidative metabolism by cyanide poisoning (Westerblad & Allen, 1991), or by inhibition of glycoly iodoacetic acid (Ruff, 1996). In the case of iodoacetate inhibition of glycolysis, electrical stimulation produces a sufficient increase in [cause contractures. These studies also demonstrated that elevated $[Ca^{2+}]_i$ does not depend on depletion of ATP, that might impair the Ca^{2+}- activity of the sarcoplasmic reticulum (SR). Also, efflux of Ca^{2+} from the ryanodine channel is virtually absent when the pH falls below is therefore unlikely to contribute to elevated $[Ca^{2+}]_i$ (Ma *et al.* 1988).

Sarcoplasmic Ca^{2+}-ATPase function

The uptake of Ca^{2+} from the cytoplasm and its transport into the lumen of the SR involves an ATPase protein with two distinct re cytoplasmic region, which includes the domains involved in catalysis, and a transport domain containing the Ca^{2+}-binding sites involve translocation of the ion through the membrane (Clarke *et al*, 1989). During catalysis, the ATPase protein cycles between two conformations: a high Ca^{2+} affinity-low saturation (E1) state that binds to cytoplasmic Ca^{2+}; and a low affinity-high saturation state that the bound Ca^{2+} into the lumen of the SR (E2) (de Meis & Vianna, 1979). The sequence of events that lead to transport of Ca^{2+} into the however reversible, and can therefore mediate the efflux of Ca^{2+} from the SR into the sarcoplasm. In the presence of substrate (ADP and P can be coupled to the resynthesis of ATP (de Meis & Tume 1977; Tanford, 1984) but can also proceed without coupling to ATP synth Meis and Inesi, 1992). Under normal circumstances, the low affinity of the E2 site will ensure that the rate of the backwards reaction wil in spite of high intraluminal $[Ca^{2+}]$, but it is evident that the net sarcoplasmic $[Ca^{2}]$ will be defined by the rate constants of the forw backward reactions of the Ca^{2+}-ATPase.

Can the kinetics of SR Ca^{2+}-ATPase account for increased $[Ca^{2+}]_i$ and accelerated glycolysis following electrical stimulation?

In order for there to be an increase in $[Ca^{2+}]_i$, the kinetics of the reverse reaction (Ca^{2+}-efflux) need to be enhanced relative to the forward re Other than by an increase in SR $[Ca^{2+}]$, an unlikely consequence of electrical stimulation, experimental evidence has shown that efflux stimulated by increased [ADP], particularly when SR $[Ca^{2+}]$ are high (Pick & Bassilian, 1983; Inesi & de Meis, 1989). Under condi intraluminal $[Ca^{2+}]$ between 20-40 mM, the rate of Ca^{2+} efflux is doubled by an increase in [ADP] from 1 to 100 μM, an effect attributed p to an increase in the proportion of nucleotide bound intermediates of the enzyme (Inesi & de Meis, 1989). Unfortunately, chemical measu of [ADP] in muscle are difficult because the majority of ADP present in the cell is bound to myofibrillar proteins, particularly actin. problematic, [ADP] in muscle are too low to be detected directly by ^{32}P-NMR. However, free [ADP] can be calculated from the equi constant of the CK reaction:

$$k=[Creatine][ATP]/[ADP][CP][H^+]$$

where $k=1.66x10^9$ at an approximated $[Mg^{++}]$ of 2.5 mM (Lawson & Veech, 1979), and a measured concentration of 29 μM/g wet w creatine. Concentrations were converted from wet weight to intracellular water using the volume fraction 0.58 g intracellular water, ex sarcoplasmic reticulum and mitochondria, per g wet muscle (Baylor et al., 1982).

The ADP concentrations at pH 6.2, calculated from NMR recording in lamb muscle are shown in Table 1. It is evident that electrical stim results in significantly lower CP, and higher ADP concentrations than in unstimulated muscle at the equivalent pH ($P<0.01$). Mor relationship was evident between ADP concentrations at pH 6.2 and the rate of ATP turnover at that pH in muscle (Figure 4).

Table 1.
Metabolic status of lamb m. semitendinosus at pH 6.2

	Unstimulated	Stimulated
ATP (μmol/g)	3.8 (s.e. 0.4)	2.9 (s.e. 0.6); $p>0.05$
CP (μmol/g)	2.9 (s.e. 0.2)	0.9 (s.e. 0.07); $p<0.01$
Calculated ADP (μM)	0.37 (s.e. 0.03)	0.76 (s.e. 0.04); $p<0.01$

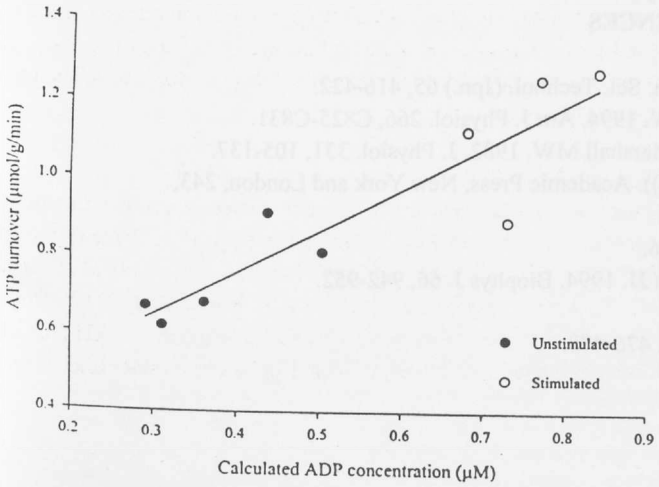

Figure 4. Relationship between calculated [ADP] and ATP turnover

Myofibrillar sensitivity to Ca^{2+}

While changes in the kinetics of sarcoplasmic Ca^{2+}-ATPase may account for the observed rise in $[Ca^{2+}]_i$ following stimulation of anoxic muscle, it still remains to be determined whether this is a sufficient explanation for the accelerated rate of pH fall in electrically stimulated muscle. Recently, Ruff (1996) has suggested that the myofibrillar sensitivity to Ca^{2+} is enhanced in the presence of elevated [ADP] (although other have not found such an effect, for example Cooke & Pate, 1985), resulting in a significant increase in the tension-Ca^{2+} relationship of single mouse muscle fibres. On the basis that tension development and ATPase activity are necessarily related (Barany, 1967), these studies suggests the possibility that ATP turnover, and therefore pH fall in the anoxic muscle, will be increased by both higher resting $[Ca^{2+}]_i$ and greater sensitivity of the actomyosin-ATPase to the increased $[Ca^{2+}]_i$.

Role of P_i in post mortem metabolism

Inorganic phosphate concentrations accumulate during the post mortem period as a result of the hydrolysis of CP and of ATP. These changes in $[P_i]$ can have important influences on post mortem energy metabolism through changes in the control of $[Ca^{2+}]_i$ and in the levels of ATPase activity.

Increasing $[P_i]$ enhances the uptake capacity of Ca^{2+} into the sarcoplasmic reticulum (Newbold & Tume, 1981; Inesi & de Meis, 1989), and also reduces the release of Ca^{2+} from the sarcoplasmic reticulum (Westerblad & Allen, 1991; Fryer *et al.*, 1995). Fryer *et al* (1995) proposed that these consequences of increased $[P_i]$ could be accounted for by the accumulation of P_i within the sarcoplasmic reticulum, where it complexes with Ca^{2+} and thus reduces free $[Ca^{2+}]$ in the sarcoplasmic reticulum; indeed, the solubility product of calcium phosphate can be exceeded within the range of $[P_i]$ found in post mortem, resulting in precipitation of Ca^{2+}. Therefore, increased $[P_i]$ reduces sarcoplasmic reticular $[Ca^{2+}]$, which stimulates uptake and inhibits efflux of Ca^{2+}, and so contribute to lower $[Ca^{2+}]_i$.

Increasing $[P_i]$ also reduces actomyosin ATPase activity (Cooke & Pate, 1985; Parkhouse, 1991) and inhibits myofibrillar Ca^{2+} sensitivity (Fryer et al., 1995). Similar effects on myofibrillar ATPase activity and Ca^{2+} sensitivity are produced by a drop in pH (Blanchard *et al.*, 1984; Parkhouse, 1991). From the standpoint of understanding the rate of post mortem ATP turnover and pH decline, the effect of increased $[P_i]$ will be to oppose the influence of increasing [ADP] proposed earlier, by increasing uptake and reducing release of Ca^{2+} from the sarcolemma, and reducing the myofibrillar sensitivity to Ca^{2+}.

CONCLUSIONS

Electrical stimulation of carcasses to accelerate pH fall offers important processing advantages, although, particularly in the case of low voltage stimulation, the accelerated pH fall subsequent to the stimulation can be variable (Eikelenboom *et al*, 1985). Not unexpectedly, ^{32}P-NMR data found that the rate of ATP turnover is increased following electrical stimulation and therefore drives the faster rate of pH decline. The link between the rate of ATP turnover and the calculated free ADP concentrations is proposed to be ADP-induced efflux of Ca^{2+} from the sarcoplasmic reticulum. Electrical stimulation produces the conditions necessary for increased [ADP] through the disproportionate use of CP relative to glycolysis during the stimulation period, resulting in a lower [CP] to $[H^+]$ ratio and thus conditions that favour ADP accumulation. Without stimulation, [ADP] are lower, and the accumulation of P_i, together with the decline in pH, act to maintain low $[Ca^{2+}]_i$ and reduce myofibrillar ATPase activity.

Variability in the rate of pH fall subsequent to electrical stimulation could be attributed to differences in the resynthesis ratio (ATP resynthesised by PCr / ATP resynthesised by glycolysis) during the stimulation, leading to different post-stimulation ADP concentrations. The resynthesis ratio is likely to be influenced by the electrical parameters used for stimulation, and by the metabolic state of the muscle at the time of stimulation, particularly by [CP].

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