METABOLIC EFFECTS OF TOURNIQUET ISCHAEMIA STUDIED
BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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A model of tourniquet ischaemia was developed in the hind limb of the rat, and the metabolic changes
that occurred in the calf muscles were monitored by the non-invasive technique of phosphorus-31 nuclear
magnetic resonance spectroscopy. During ischaemia the intramyocellular pH became acidic as the level of
phosphocreatine declined and that of inorganic phosphate rose. Phosphocreatine was no longer detectable
after approximately 2 hours and ATP was depleted after approximately 3.5 hours. Metabolic recovery was
rapid (1 hour) if ATP was present when the tourniquet was released but was prolonged (3 or more hours) if
ATP was depleted. Hourly release of the tourniquet for 10 minutes ensured the maintenance of ATP and
rapid metabolic recovery. Release for intervals of only 5 minutes did not have the same protective effect and
in fact worsened tissue pH during the period of tourniquet ischaemia. Heparin and corticosteroids were
without effect during and after periods of tourniquet ischaemia.

The pneumatic tourniquet was introduced in 1904 by
Cushing to maintain a bloodless field while operating on
the extremities. Since then its use has become almost
routine, but opinion is still divided as to the length of
time this potentially destructive instrument can be applied (Bruner 1970; Dahlbäck 1970; Flatt 1972;
Klenerman et al. 1980).

Many factors have been implicated in the develop-
ment of post-tourniquet complications but the length of
the ischaemic episode is one of the most important
(Wilgis 1971 and 1974). Two hours is generally accepted
as safe for the human upper limb (Flatt 1972); hourly
deflation of the tourniquet has been recommended if it is
necessary to exceed this time-limit (Bruner 1951; Bunnell
1956; Flatt 1972). However, experimental evidence to
support these clinical aphorisms is sparse.

The release of intracellular enzymes to the extra-
cellular space, together with pH changes of the extra-
cellular fluid, are considered by some to be the earliest
biochemical signs of ischaemic cellular damage. Accord-
ingly, measurements have been made on venous blood
withdrawn during and after the use of pneumatic tourniquets in man (Solonen et al. 1968; Wilgis 1971,
1974; Klenerman 1980) and in animals (Chiu, Wang and
Blumenthal 1976; Miller et al. 1978). These investigations
were based on the premise that chemical changes in
venous blood accurately reflect cellular metabolism.
Indeed, it was Wilgis (1971) who stated that “measurement
of pH constituted the best method of assaying the
degree of cellular acidosis”.

That ischaemia affects cell metabolism is incontro-
vertible, but that the degree of change and its reversal
after tourniquet release are accurately reflected in the
biochemistry of venous blood is questionable. Wilgis
(1971) drew attention to the role of the blood buffer
system in diminishing the degree of acidosis and hypercapnia, and Haljamäe and Enger (1975) reported
that blood-gas measurements did not adequately reflect
tissue metabolism when perfusion was abnormal. Simi-
larly, arteriovenous shunting which permits little or no
oxygen diffusion between capillaries and tissue has been
described after tourniquet release (Wilgis 1971; Miller
et al. 1978; Edfeldt and Thomson 1980). If accurate data
are to be obtained on the metabolic changes that occur
during and after ischaemia, indirect techniques must be
replaced by those that directly monitor cellular events.

Micro-electrodes have been tried (Pennig and Brug
1982) but these are difficult to use, invasive and give
information solely about intracellular pH. Fortunately
high-resolution phosphorus nuclear magnetic resonance
spectroscopy (31P NMR) has recently been introduced as
a painless and non-invasive method of studying the
biochemistry of muscle (Ross et al. 1981). This technique
is based upon the interaction between phosphorus nuclei
within tissues situated in a magnetic field and radio-
frequency energy. Signals (resonances) that reflect the
intracellular levels of phosphocreatine, adenosine tri-
phosphate (ATP), and inorganic phosphate can be
synchronously recorded within a few minutes; intra-
cellular pH can then be derived from the spectrum (Moon
and Richards 1973). (For reviews of this technique in
biological research see Radda and Seeley 1979; Gadian
and Radda 1981; for clinical applications see Radda
et al. 1982; Newman et al. 1982; Newman and Radda
We have used this non-invasive technique (based on direct monitoring of high-energy phosphate metabolism) to determine the effects of tourniquet ischaemia on the metabolism of skeletal muscle.

2. The tourniquet was applied to 5 rats for 3 hours, but released hourly for 5 minutes.
3. The tourniquet was applied to 5 rats for 3 hours, but released hourly for 10 minutes.
4. Heparin (mucous) (1.5 mg kg\(^{-1}\)) was injected intraperitoneally into 5 rats 1 hour before the tourniquet was applied and kept in position for a continuous period of 3 hours. This dose has been previously shown to increase the blood-clotting time significantly (Paletta, Willman and Ship 1960).
5. Prednisolone sodium phosphate (0.2 mg kg\(^{-1}\)) was injected intraperitoneally into 5 rats 1 hour before application of a tourniquet which was kept in position for a continuous period of 3 hours.
6. The tourniquet was applied to 5 rats and kept in position for 5 hours. During that time spectra were recorded from the contralateral control limb.

**Spectroscopy.** \(^{31}\)P NMR spectra were recorded from the experimental limbs at 10-minute intervals during and after the ischaemic episode. A TMR-32 spectrometer (Oxford Research Systems) was used. This incorporated a 1.89 Tesla superconducting magnet with a horizontal bore of 20 cm. During the spectroscopy the rat was secured in the probe in a supine position with its hind limb extended; an oval two-turn surface coil (25 \(\times\) 15 mm in diameter, and wound from 1 mm diameter insulated copper wire) was placed beneath the calf to detect signals (Ackerman et al. 1980).

**METHOD**

Male Sprague Dawley rats (weighing between 200 and 250 g) were used. They were caged individually and fed a standard laboratory diet of pellets with an ad libitum supply of water.

All experiments were conducted under general anaesthesia (45 mg of pentobarbitone sodium per kilogram of body weight injected intraperitoneally). In those animals in which the periods of ischaemia and expected metabolic recovery were to be prolonged, a 16FG catheter was inserted into the peritoneal cavity. This facilitated the administration of further doses of anaesthetic without moving the animal from the spectrometer.

Plastic cable-ties (with a width of 5 mm) were used as tourniquets, and were applied at the mid-thigh level to one of the hind limbs of each rat (the side being determined from a set of random numbers). A special device was used to fasten the ties to the same tension each time.

The rats were treated by one of the following protocols:

1. The tourniquet was applied to 5 groups of 5 rats and kept in position for a continuous period of either 1, 2, 3, 4 or 5 hours.

2. The tourniquet was applied to 5 rats for 3 hours, but released hourly for 5 minutes.
3. The tourniquet was applied to 5 rats for 3 hours, but released hourly for 10 minutes.
4. Heparin (mucous) (1.5 mg kg\(^{-1}\)) was injected intraperitoneally into 5 rats 1 hour before the tourniquet was applied and kept in position for a continuous period of 3 hours. This dose has been previously shown to increase the blood-clotting time significantly (Paletta, Willman and Ship 1960).
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RESULTS

All animals survived the procedure but the experimental limb rapidly became swollen after removal of the tourniquet.

Control limb. Spectra recorded from the contralateral control limb did not show any variation during and after a period of 5 hours continuous ischaemia.

Ischaemic limb. After the tourniquet had been tightened the phosphocreatine signal decreased and that of inorganic phosphate increased (Fig. 1); the ratio of phosphocreatine to inorganic phosphate after 30, 60 and 90 minutes of tourniquet ischaemia was 0.8 ± 0.02 (SEM): 1, 0.4 ± 0.01:1, and 0.2 ± 0.01:1 respectively (normal 15.0 ± 0.05:1, n = 20). The ratio of phosphocreatine to ATP at 30, 60 and 90 minutes was 1.3 ± 0.09:1, 0.8 ± 0.05:1, and 0.5 ± 0.04:1 respectively (normal 2.9 ± 0.1:1, n = 20); little change was noted in the ATP signal until that of phosphocreatine was no longer recorded.

Intracellular pH remained almost constant for the first 15 minutes of ischaemia but thereafter declined linearly (Fig. 2). Only after prolonged ischaemia (more than 4 hours) did the pH fall to lower than 6.0.

A signal indicating the presence of phosphomonoesters (sugar phosphates) was consistently recorded after approximately 75 minutes of ischaemia.

The phosphocreatine signal was not detectable after 115 ± 5 minutes of ischaemia (when the pH was 6.62 ± 0.02), and ATP after 190 ± 5 minutes of ischaemia (when the pH was 6.25 ± 0.02).

When the tourniquet was released within 3 hours, ATP was still present, and the resynthesis of phosphocreatine and the subsequent recovery of intracellular pH was rapid, i.e. within 1 hour (Fig. 3). When its release was delayed for longer than 3 hours, ATP was not detectable, and metabolic recovery was prolonged (Fig. 4).

Though relief of the ischaemia was associated with an immediate reduction in the signal intensity of inorganic phosphate and an increase in that of phosphocreatine, tissue pH continued to decline for a variable period before returning to normal (Fig. 2). The phosphomonoester signal disappeared approximately 20 to 30 minutes after the tourniquet was released.

Release of the tourniquet for 10 minutes at hourly intervals produced different metabolic changes. After 3 hours of intermittent ischaemia the pH of the tissues was 6.55 ± 0.02 (Fig. 5); levels of ATP were normal and some phosphocreatine was present. After 3 hours of continuous ischaemia the pH was 6.30 ± 0.02, and neither phosphocreatine nor ATP were detected. Furthermore, the additional fall in pH after release of the tourniquet was by 0.1 over 20 minutes with 3 hours of intermittent ischaemia compared with a drop of 0.35 over 30 minutes after the same period of continuous ischaemia (P < 0.01). Recovery occurred within 1 hour with intermittent ischaemia as opposed to 3.5 ± 0.2 hours with continuous ischaemia (P < 0.01).

Release of the tourniquet for only 5 minutes at hourly intervals had different effects. After 3 hours of intermittent ischaemia the pH was 6.10 ± 0.01 and the signal of phosphocreatine was not recorded. In 4 of the 5
rats ATP was still present and recovery of pH took 2.5 ± 0.1 hours, but in the remaining rat ATP was depleted and return to normal pH took longer.

Neither prednisolone nor heparin was found to affect the metabolic changes during or after tourniquet ischaemia.

This study using non-invasive $^{31}$P NMR has shown that tourniquet ischaemia is characterised by a progressive decrease in the level of phosphocreatine and an associated increase in that of inorganic phosphate must be replaced by others that directly monitor cellular biochemistry.

DISCUSSION
The histological changes produced in skeletal muscle by ischaemia have been documented (Solonen and Hjelt 1968; Wilgis 1974; Dahlbäck 1970; Tountas and Bergman 1977), but the associated biochemical changes have not been adequately defined. This is because reliance has been placed upon techniques that measure changes in the extracellular compartment only. If accurate data are to be obtained on the metabolic changes that occur during and after ischaemia such traditional methods (Fig. 1). This is accompanied by intracellular acidosis and the accumulation of phosphomonoesters (probably from stored glycogen). Only when phosphocreatine is depleted is a significant change seen in the signal intensity of ATP.

Such metabolic effects are to be expected during tourniquet ischaemia—a procedure that prevents tissue oxygenation and the clearance of metabolites. Nonetheless, the rate of the observed changes was slow in comparison to those observed during exercise. For
example, Radda et al. (1982) and Taylor et al. (1983) have indicated that the level of phosphocreatine may fall by 50% after 5 minutes of exercise. During tourniquet ischaemia, the skeletal muscle is at rest and the only expenditure of energy is for basal metabolism; thus, in spite of the circulatory arrest, the biochemical deterioration is relatively slow.

The decline in tissue pH and phosphocreatine was almost linear (apart from an initial lag-period which probably reflected the presence of residual oxygen within the tissues at the time the tourniquet was applied); similar findings have been reported previously (Solonen et al. 1968; Wilgis 1974; Thulborn 1981; Pennig and Brug 1982). Haljamäe and Enger (1975) reported that the rate of decline of phosphocreatine decreased with increasing tourniquet time and felt this was due to the protective effect of the loss of temperature within the ischaemic limb; however, they made only two measurements during 90 minutes of ischaemia.

that during the actual period of ischaemia; therefore hydrogen ions must have been produced. Neither gross muscle-contraction nor fibrillation was observed and the most likely explanation is that the rapid resynthesis of phosphocreatine in the reperfused tissue generated hydrogen ions thus:

creatine + ATP ⇌ phosphocreatine + ADP + H⁺
(modified after Veech et al. 1979). Calculations based upon a resting phosphocreatine concentration of 25 mmol per kilogram of wet weight and a tissue-buffering capacity of 22 mmol per kilogram of wet weight per pH unit (Taylor et al. 1983) showed that the observed decline in pH after removal of the tourniquet was compatible with the number of hydrogen ions theoretically produced during the above reaction.

A further factor for consideration is the rate of removal of hydrogen ions by the circulation, since should this exceed the rate of production acidosis may ensue.

![Graph showing the effect of hourly release of the tourniquet for periods of 5 and 10 mins on intramyocellular pH compared with continuous ischaemia. Each point represents the mean measurement of pH from 5 rats; the standard error of the mean for each point was 0.02 pH units during the ischaemic period and 0.04 pH units during the recovery phase.](image_url)

Of particular interest was the fact that the tissue pH continued to decline after removal of the tourniquet, which may have been related to continuing ischaemia. For example, Strock and Majno (1969) applied tourniquets to the hind limbs of rats for 2.5 hours and investigated vascular patency using a carbon-black perfusion technique. They observed that flow did not return immediately to all areas, taking approximately 1.75 hours to return to normal.

In our study the rate of decline of pH in the initial period after release of the tourniquet was greater than Several authors, including Wilgis (1971), Miller et al. (1978) and Edfeldt and Thomson (1980) have demonstrated arteriovenous shunting after tourniquet release and this may be a further factor in precipitating tissue acidosis.

The biochemical determinant of the speed of metabolic recovery after tourniquet release was the level of ATP; rapid recovery always occurred in its presence (Fig. 3), but not in its absence (Fig. 4). This point was also indicated by Thulborn (1981) in a pilot study of ischaemia of skeletal muscle. Tissue pH at the time of
tourniquet release was not such a critical factor.

Similar effects have been noted by Radda (personal communication, 1983) during a NMR investigation of human muscle during exercise. Normally, ATP was maintained at a more or less constant level and the recovery of phosphocreatine, inorganic phosphate and pH occurred rapidly during the resting period. However, in a small proportion of subjects, ATP was partially depleted and spectra recorded up to 30 minutes after exercise failed to show its resynthesis.

In an attempt to minimise the ischaemic damage caused by tourniquets it is common practice to deflate the tourniquet intermittently. Since it has been shown that the metabolic changes that occur in venous blood after an hour of tourniquet ischaemia take approximately 5 minutes to revert to normal (Wilgis 1971; Miller et al. 1978), it has been suggested that hourly release for only a few minutes would be sufficient to prevent damage.

This study has shown that the length of the so-called "breather" period is of vital importance. Periods of 10 minutes prevented the depletion of ATP and hence, during 3 hours of intermittent ischaemia, the metabolic demands for chemical energy could be met. This can be compared with the results of Chiu et al. (1976) which demonstrated that hourly release of the tourniquet for 15 minutes prevented the release of creatine phosphokinase into the extracellular fluid. This NMR study indicated that intervals of 5 minutes did not prevent ATP depletion and, in addition to causing a deterioration of tissue pH, did not necessarily shorten the recovery time.

On the basis of these results it is suggested that breather periods (if considered necessary) should be of at least 10 minutes duration. The exact interval, however, should be decided by the surgeon and the anaesthetist at the time of operation since the additional blood-loss and the patient's ability to withstand a prolonged operation must be taken into consideration.

Pharmacological techniques including the administration of heparin (Paletta 1960; Strock and Majno 1969) and prednisolone (Edfeldt and Thomson 1980) have also been used in an attempt to minimise the damage to muscle caused by ischaemia. Heparin was found to lessen post-tourniquet oedema in dogs subjected to 5 hours of ischaemia, whether it was injected before the tourniquet was applied or after its removal (Paletta et al. 1960). These results are difficult to understand since if heparin is effective by preventing intravascular coagulation, as suggested, the benefits should have been seen only when the drug was administered before the tightening of the tourniquet. Furthermore, because of the marked increase in fibrinolytic activity of the blood which occurs when the tourniquet is released (Klenerman et al. 1977), it has been suggested that the use of tourniquets is associated with an unaltered or decreased risk of deep venous thrombosis (Kroese and Stiris 1976). This has recently been confirmed clinically (Simon et al. 1982), and in the hind limb of rats (Strock and Majno 1969).

The rationale for the use of steroids to minimise ischaemic tissue damage is based upon the suggestion that it minimises the vascular shunt that occurs after tourniquet release (Wilgis 1971; Miller et al. 1978; Edfeldt and Thomson 1980). However, the improvement is only marginal and Edfeldt and Thomson could not recommend its routine use.

This non-invasive study using $^{31}$P NMR failed to demonstrate any effect of heparin or steroids on the metabolic changes that occurred during or after periods of tourniquet ischaemia, but intermittent release of the tourniquet was beneficial providing the period of release was of at least 10 minutes' duration.

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REFERENCES


