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Real time measurement of intramuscular pH during routine knee arthroscopy using a tourniquet

A PRELIMINARY STUDY

D. R. W. MacDonald,
D. W. Neilly,
K. E. Elliott,
A. J. Johnstone

From Woodend
Hospital, Aberdeen, UK

Aims

Tourniquets have potential adverse effects including postoperative thigh pain, likely caused by their ischaemic and possible compressive effects. The aims of this preliminary study were to determine if it is possible to directly measure intramuscular pH in human subjects over time, and to measure the intramuscular pH changes resulting from tourniquet ischaemia in patients undergoing knee arthroscopy.

Methods

For patients undergoing short knee arthroscopic procedures, a sterile calibrated pH probe was inserted into the anterior fascial compartment of the leg after skin preparation, but before tourniquet inflation. The limb was elevated for three minutes prior to tourniquet inflation to 250 mmHg or 300 mmHg. Intramuscular pH was recorded at one-second intervals throughout the procedure and for 20 minutes following tourniquet deflation. Probe-related adverse events were recorded.

Results

A total of 27 patients were recruited to the study. Mean tourniquet time was 21 minutes (10 to 56). Tourniquet pressure was 300 mmHg for 21 patients and 250 mmHg for six patients. Mean muscle pH prior to tourniquet inflation was 6.80. Muscle pH decreased upon tourniquet inflation, with a steeper fall in the first ten minutes than for the rest of the procedure. Change in muscle pH was significant after five minutes of tourniquet ischaemia ($p < 0.001$). Mean muscle pH prior to tourniquet release was 6.58 and recovered to 6.75 within 20 minutes following release. No probe related adverse events were recorded.

Conclusion

It is possible to directly measure skeletal muscle pH in human subjects over time. Tourniquet ischaemia results in a decrease in human skeletal muscle pH over time during short procedures.

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Keywords: Ischaemia, pH monitoring, Arthroscopy, Tourniquet, Muscle physiology

Article focus

- We aimed to determine if it is possible to directly measure intramuscular pH in human subjects over time.
- We aimed to measure the intramuscular pH changes resulting from tourniquet ischaemia in patients undergoing short, routine arthroscopic knee procedures.

Key messages

- We confirmed that it is possible and acceptable to patients to directly monitor skeletal intramuscular pH in real time in human subjects using a pH probe.
- The application of a limb tourniquet with subsequent development of ischaemia resulted in a fall in distal intramuscular pH during short arthroscopic procedures.

Correspondence should be sent to David R. W. MacDonald; email: david.macdonald7@nhs.scot

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- This is a preliminary study, and through future research it may be possible to correlate changes in intramuscular pH during procedures that use a tourniquet with postoperative symptoms and function.

Strengths and limitations

- This study is novel as no other study has been published describing the direct measurement of intramuscular pH in human subjects over time following tourniquet application.
- This study was limited as the procedures and therefore tourniquet times included in our study were short. We are therefore unable to comment on whether the pattern of pH decline seen would continue over longer procedures.
- The sterilization procedure for the pH probe resulted in variation in starting pH and wide standard deviation of pH measurements; the method of sterilization will be altered in future studies to avoid this problem.

Introduction

The application of a tourniquet has been shown to significantly improve visibility during arthroscopic knee surgery.¹ The most frequently performed arthroscopic procedures, such as medial meniscectomy,² have short tourniquet times, often under 20 minutes. However, complex arthroscopic procedures have become more commonplace in recent years leading to increased tourniquet times, sometimes over 90 minutes.

Despite the technical benefits of tourniquet application, there are a number of known potential complications of tourniquet use. Several studies have demonstrated that tourniquet use for arthroscopic knee surgery may result in muscle atrophy and reduced quadriceps and hamstring function.^{3,4}

Tourniquet application may also result in pain at the site of the tourniquet during surgery, thought to be mediated by a cutaneous neural mechanism due to localized compression.⁵ In extreme circumstances “post tourniquet syndrome” may occur, in which the affected limb is swollen, stiff, pale, and weak for one to six weeks following tourniquet application.⁶

It is likely that many of these potential complications are the result of the ischaemic effect of tourniquets on the lower limb, although direct compression of the thigh could also be a contributing factor. Skeletal muscle is a highly metabolically active tissue. Even at rest the turnover of adenosine triphosphate (ATP) is calculated to be close to 35 $\mu\text{mol}\cdot\text{kg}^{-1}$ muscle.⁷ When oxygen delivery and availability are abundant, ATP is primarily resynthesized through oxidative phosphorylation of fatty acids via mitochondrial respiration. However, when oxygen availability is below that required for oxidative phosphorylation, the energy required to resynthesize ATP is produced using glycolysis (anaerobic respiration). Pyruvate, the end product of glycolysis, is converted into

lactic acid resulting in the accumulation of H^+ ions intracellularly and extracellularly, and consequently a fall in skeletal muscle pH. Heppenstall et al⁸ demonstrated that tourniquet application and subsequent ischaemia results in a fall in skeletal muscle pH. This research was undertaken using timed biopsy specimens from the anterolateral compartment of the legs of beagle dogs following proximal tourniquet application, and showed an almost linear fall in pH with tourniquet time.

In human subjects, pH monitoring has been used for several decades but primarily for monitoring oesophageal pH in patients with gastroesophageal reflux disease.⁹ Invasive pH monitors have been used successfully to measure changes in the myocardium during cardiac bypass,¹⁰ and to monitor the pH of brain tissue¹¹ and the intestinal mucosa.¹² They have also been successfully used to measure the pH of skeletal muscle during shock resuscitation.¹³ However, there have been no studies published describing the measurement of falling intramuscular pH over time following tourniquet application in human subjects.

The aims of this preliminary study were to confirm that it is possible to directly measure intramuscular pH in real time in human subjects, and to measure intramuscular pH changes from tourniquet ischaemia in patients undergoing simple and short knee arthroscopic procedures.

Methods

Over a five-month period, patients admitted to our centre for routine arthroscopic knee surgery were invited to participate in our study. Patients were excluded if they were under 16 years of age or were unable to speak and understand English. The trial was approved by our local ethical review committee.

A 1.5 mm glass probe (Mettler Toledo) made of durable, heat strengthened, fracture-proof glass was used to monitor the pH within the anterior muscle compartment of the appropriate leg of each patient for this study. The probe was connected to the Flexilog 2020 dual channel ambulatory pH recorder (Oakfield Instruments, UK). This allowed continuous recording of pH (range from 0 to 10), accurate to one decimal place, at intervals of one second. The recorder had a marker facility to allow events to be registered during the pH recording. This was used to mark the inflation and deflation of the tourniquet. The probe and the monitor were connected to an external reference electrode placed on the patient's skin outside the operative field.

The pH monitor was calibrated on each patient in the ward prior to arrival in theatre using the monitor's in-built calibration system, which uses pH buffers of 7.0 and 1.1. A circuit was created with the monitoring unit, the probe, and the patient using an external reference electrode (ECG pad) and by placing the tip of the probe and the patient's finger simultaneously within a container of buffer solution. This process was repeated using the

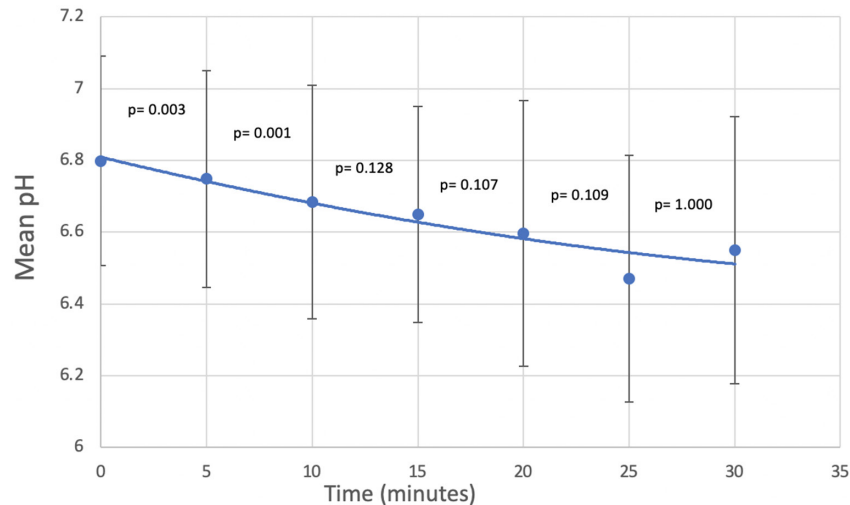


Fig. 1

Intramuscular pH changes during tourniquet ischaemia. The p-values demonstrate significance of difference in pH fall between each five-minute interval (Wilcoxon ranked pairs test).

second buffer solution ensuring accurate readings and completing system calibration. Following calibration, the probe was disconnected and sterilized to surgical standards in a Tristel 700 sterilization bath (Tristel Solutions, UK). The probe was submerged in the bath for ten minutes, as recommended in the Tristel guidelines, and was then rinsed in sterile N-saline and placed on a prepared sterile trolley immediately prior to insertion. Once general anaesthesia had been induced, a tourniquet was placed on the appropriate thigh, but not inflated. An external reference electrode was placed on the lateral thigh of the operative limb, and the surgical site was prepared and draped in the individual surgeon's routine fashion.

A 14-gauge Adsyte intravenous cannula was then inserted into the anterior compartment of the leg, approximately 2 cm lateral and 4 cm distal to the tibial tubercle, at a safe site away from the position of impending incisions, at an angle of approximately 30° to the skin, parallel with the muscle fibres. Upon penetration of the fascia the cannula was levelled out and further advanced to its limit. The needle was then removed and the probe inserted through the lumen of the plastic sheath to a distance of approximately 1 cm beyond the tip of the sheath into the muscle belly. The cannula was then withdrawn over the probe, the probe secured with an Opsite dressing (Smith & Nephew Healthcare, UK), and the remainder of the probe held out of the operative field with a loosely applied six-inch crepe dressing. The probe and reference electrode were then connected to the Flexilog recorder and the leg was elevated for three minutes prior to tourniquet inflation to 250 mmHg or 300 mmHg.

The limb pH was recorded immediately before tourniquet inflation and at one-second intervals after inflation. Blood pressure, peripheral oxygen saturation using a finger probe, and end tidal carbon dioxide (ETCO₂) were

also recorded at intervals of five minutes during the procedure using the Kritikon Dynamap 1846 SX, the Ohmeda Biox 3740 pulse oximeter, and the Captronic Ultra ETCO₂ monitor, respectively. Following tourniquet deflation, the probe remained in situ for 20 minutes during which time the muscle pH, blood pressure, and peripheral oxygen were monitored.

The probe was easily removed with minimal discomfort in the recovery room and a small gauze dressing applied to the puncture wound. The probe was then wiped clean and immediately placed into a buffer solution of 7.0 as part of a circuit identical to that used for initial calibration. A reading was obtained after one minute, allowing any drift in the monitoring system to be measured. Information about any pH probe-related adverse effects, such as bruising and infection at the site of insertion, were asked at routine patient follow-up six weeks following surgery.

Statistical analysis. Statistical analysis of the results was completed using SPSS for Macintosh version 26 (IBM, USA). Non-parametric statistical tests were used due to the skewed distributions of the samples. Wilcoxon matched pairs test was used to analyze the change between mean starting pH and mean pH prior to tourniquet deflation. Wilcoxon ranked pairs test was used to analyze the pH changes at five-minute intervals to delineate if there was a significant change between each timepoint.

To investigate if patient age or ETCO₂ had any effects on baseline muscle pH, ischaemic pH change, or pH recovery, Spearman's rho correlations were used. The effects of sex, tourniquet pressure (250 mmHg vs 300 mmHg), and leg side on baseline pH, ischaemic pH change, and pH recovery were determined using the Mann-Whitney U test.

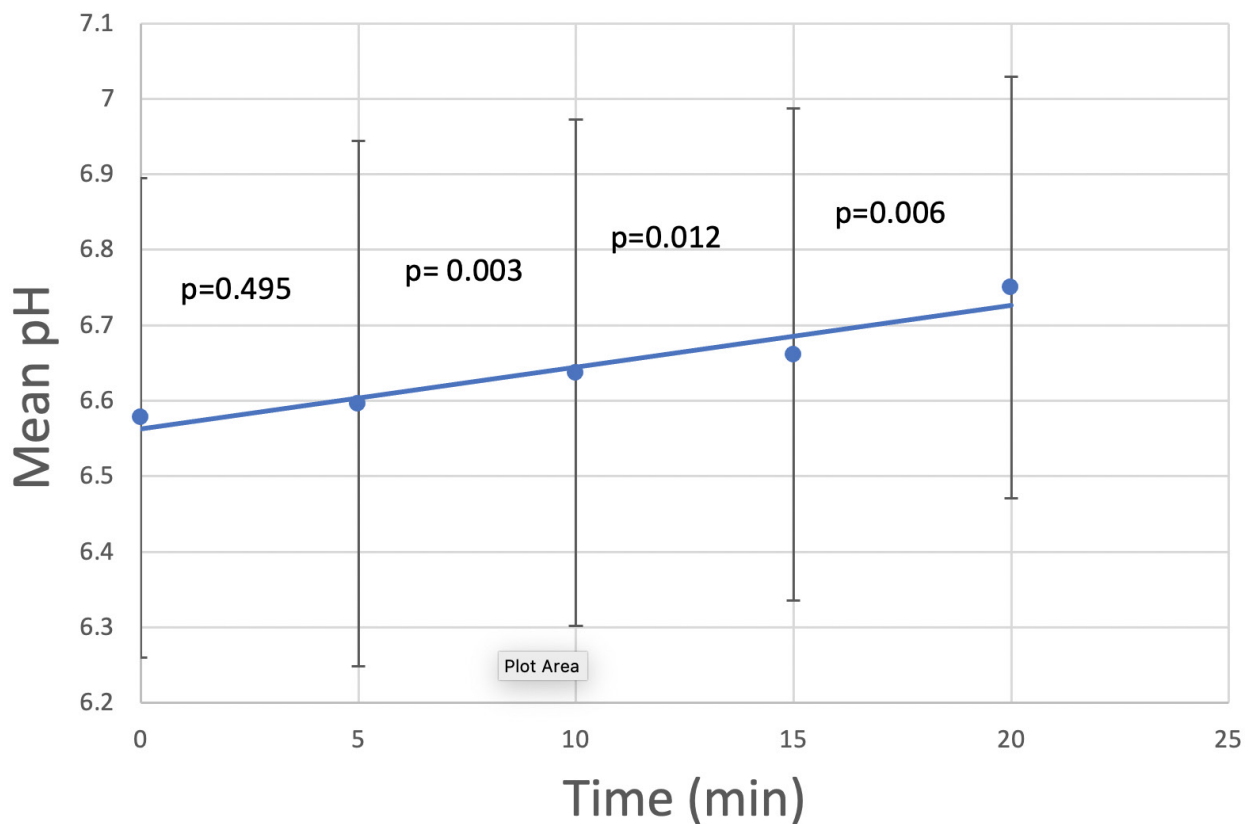


Fig. 2

Intramuscular pH changes following release of the tourniquet. The p-values demonstrate significance of difference in pH recovery between each five-minute interval (Wilcoxon ranked pairs test): 0 to 5 mins, $p = 0.495$; 5 to 10 mins, $p = 0.003$; 10 to 15 mins, $p = 0.012$; 15 to 20 mins, $p = 0.006$.

Results

In total, 27 patients were recruited to the study over a five-month period. Of these, 19 patients were male and eight were female. Mean patient age was 40 years (17 to 75). Mean tourniquet time across all patients was 21 minutes (10 to 56). A tourniquet pressure of 300 mmHg was used for 21 patients and a tourniquet pressure of 250 mmHg was used in six patients. No probe-related adverse effects were recorded during the study or at clinic follow-up review, and the protocol was acceptable to all patients.

The mean change in pH during tourniquet ischaemia is demonstrated in Figure 1. The mean muscle pH prior to tourniquet inflation was 6.80 (6.20 to 7.20). The mean muscle pH declined following tourniquet application and this was significantly different to the starting pH after just five minutes of tourniquet application ($p < 0.001$). Wilcoxon ranked pairs testing found that the decrease in pH between tourniquet inflation and five minutes, and five minutes to ten minutes, decreased significantly more than during the other five-minute intervals ($p = 0.003$ and $p = 0.001$, respectively).

The mean muscle pH prior to tourniquet deflation was 6.58 (6.15 to 7.30), which was significantly lower than the

mean starting pH ($p < 0.001$). Upon release of the tourniquet the intramuscular pH increased, as demonstrated in Figure 2. It remained significantly lower than baseline pH values until 20 minutes following removal, at which point the mean intramuscular pH was 6.75 (6.25 to 7.35).

The mean recorded value when the pH probe was placed in the pH 7.0 buffer following removal from the muscle was 7.07 (6.5 to 7.7; standard deviation (SD) 0.28), which does not represent a significant drift during recording with the exception of one aberrant reading of 6.5 ($p = 0.226$, Wilcoxon matched pairs test). Comparing the initial muscle pH and the drift pH with the Shapiro-Wilk test showed that the drift data were normally distributed, and a Q-Q plot showed there was minimal effect size. Pearson's correlation test showed that there was a statistically significant correlation between starting pH and drift pH measurements ($p = 0.002$).

Discussion

This preliminary study confirms that it is possible to directly monitor skeletal intramuscular pH in real time in human subjects using a pH probe. Patients in our study found this acceptable, and no probe-related complications were recorded. Although further work is required

Table 1. Effect of external factors on initial pH, ischaemic pH change, and pH recovery.

Variable	Age*	Sex†	T pressure†	ETCO ₂ *	Leg side†
Baseline pH	p = 0.233	p = 0.182	p = 0.062	p = 0.927	p = 0.546
Ischaemic period pH change at 10 mins	p = 0.312	p = 0.486	p = 0.031	p = 0.644	p = 0.312
Ischaemic period pH change at 15 mins	p = 0.851	p = 0.313	p = 0.019	p = 0.214	p = 0.193
Recovery	p = 0.494	p = 0.255	p = 0.090	p = 0.993	p = 0.424

*Spearman's rho correlations.

†Mann-Whitney U tests.

ETCO₂, end tidal carbon dioxide; T pressure, tourniquet pressure.

to correlate our study with clinical findings, as expected it demonstrates that the application of a limb tourniquet with subsequent development of ischaemia results in a fall in distal intramuscular pH. Over the initial 20 minutes of tourniquet application seen in this study, the fall in pH was steeper in the first five minutes and five to ten minutes than the subsequent time for the procedure, which is in keeping with results from unpublished animal models.¹³ The mean pH prior to tourniquet deflation was 6.58 and this increased to near baseline values within 20 minutes following deflation.

A number of methods to measure intramuscular pH have been described in the literature. Initially it was only possible to accurately measure pH using biopsy specimens, which was invasive and limited the number of recordings that could be taken and therefore is of limited clinical use in human subjects. The subsequent development of phosphorus-31 – magnetic resonance spectroscopy (P-NMRS) has allowed non-invasive measurement of skeletal muscle pH.¹⁴ This instrument does not give a direct reading of pH but allows pH to be derived from the shift of inorganic phosphate relative to phosphocreatine.¹⁵

Several studies have shown that it is possible to directly measure intramuscular pH using an invasive electrode inserted directly into the muscle. McKinley et al¹³ used an invasive fibre-optic pH probe to observe a reduction in the pH of the quadriceps muscle of a 37-year-old road traffic accident victim with haemorrhagic shock. Allsop et al¹⁶ directly measured muscle pH using a needle-tipped probe in the vastus lateralis for 30 minutes following a 30-second sprint in ten active subjects. Street et al¹⁷ directly measured pH in the vastus lateralis during a five-minute period of exercise using an invasive microdialysis catheter and the pH-sensitive fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF).

None of these papers document probe-related adverse effects or acceptability of the probes to patients. As the technique for pH probe insertion in our study and subsequent monitoring is similar to that commonly used for intracompartmental pressure (ICP) monitoring systems, it is reasonable to assume that complications and patient acceptability are likely to be similar for the two techniques. McQueen et al¹⁸ retrospectively reviewed data from 979 patients who had undergone ICP monitoring,

finding that none had developed complications associated with the monitoring device, and did not identify acceptability to patients as a significant problem.

No study has been published describing the direct measurement of intramuscular pH in human subjects over time following tourniquet application. The steeper initial fall in pH followed by a slower ongoing decline seen in our study is consistent with previous unpublished work in animal studies.¹⁹ However, the fact that change in muscle pH was significant after only five minutes of tourniquet ischaemia is at odds with results seen by Heppenstall et al,⁸ which suggested an initial lag period before pH fall during which time residual oxygen is used. It is possible that the elevation of patients' limbs for three minutes prior to tourniquet inflation in our study is responsible for this difference, as it may have reduced the arteriovenous gradient with less blood present within the limb, further reducing the amount of oxygen available to the muscles as well as the natural acid-base buffering properties of blood.

Our finding that, following tourniquet release, intramuscular pH increased but did not return to baseline values until 20 minutes after the tourniquet was removed, is also consistent with previous findings. Newman²⁰ demonstrated that tissue pH returned to baseline within 30 minutes following an hour of circulatory occlusion using P-NMRS in the hindlimb of a rat.

There were a number of limitations to our study. Our results demonstrated a wide range of initial starting pH values prior to tourniquet inflation. This can be explained by the sterilization process applied for the pH probes used in the study. The pH probe and the pH calibration solutions used were not manufactured sterile, so the probe had to be calibrated pre-sterilization. The Tristel sterilization solution used is strongly acidic and there may have been small residual amounts of this solution left on the probe, resulting in the observed variability in initial pH measurements. Despite this, the pH trend for each patient was strongly significant and consistent for all patients, and Pearson's correction test demonstrated a strong correlation between the initial muscle pH and final drift pH measurements. Therefore, although there is variability in the pH values measured, the pH changes over time seen in our results are consistent. This can be compared to requiring to "zero" weighing scales prior to

using them – although the initial reading on the scales may not be accurate, once zeroed, the readings thereafter change in a predictable manner with ever increasing load so the overall accuracy of the scales is maintained. This limitation also accounts for the wide SD seen at each time point in Figures 1 and 2. Although the sterilization process resulted in a wide spread of pH measurements around the mean at each time point, the trajectory of pH fall was consistent, resulting in a clear fall in mean pH over time with a highly significant difference between starting pH and pH prior to tourniquet deflation. In order to be able to use intramuscular pH measurement as an accurate tool in the future, the sterilization process used in this exploratory study will need to be refined to remove or reduce the variability of the initial pH measurement. We anticipate that in future studies with greater patient numbers, pH change will reach significance for all five-minute time intervals.

There was also heterogeneity in the tourniquet pressure used between patients. In some patients a pressure of 300 mmHg was used, while in others a pressure of 250 mmHg was used. Our analysis demonstrated that this does influence the results (Table I), and there may be some merit in using a lower tourniquet pressure for these procedures. Our study also had a relatively young cohort of patients with a mean age of 40 years (17 to 75). Our findings may therefore not be generalizable to an older population. It may be useful to conduct further research on older patients, e.g. those undergoing knee arthroplasty surgery to determine whether the same pattern of intramuscular pH is seen in those patients. Similarly, the procedures and therefore tourniquet times included in our study were short (mean 21 minutes (10 to 56)). We are therefore unable to comment on whether the pattern of pH decline seen would continue this trajectory over time, and it is assumed that the subsequent recovery in muscle pH that was observed in our study would also be extended after longer procedures, e.g. anterior cruciate ligament (ACL) reconstruction.

Longer procedures such as ACL and posterior cruciate ligament reconstruction, and nonarthroscopic cases such as revision knee arthroplasty and complex trauma, can result in much longer tourniquet times compared with the simple arthroscopic procedures described in our study. Although further work is required to confirm this, it is likely that for longer procedures there will be a much greater drop in intramuscular pH to well below 6.58. In exceptionally long procedures this may be of concern as other studies have shown that if muscle pH drops too low, this muscle damage becomes irreversible. Lange et al²¹ observed a cardiac intramural pH of 5.39 after two hours of normothermic arrest in dogs, with evidence of irreversible structural damage in muscle biopsy specimens between 60 and 90 minutes. In their biopsy specimens, irreversible cell damage was associated with an intramyocardial pH of less than 6.2. In a later study Heppenstall et al²² compared tourniquet ischaemia and an induced compartment syndrome in the hindleg of dogs,

measuring intramuscular pH with P-NMRS during tourniquet application, and taking tissue biopsies following tourniquet release to determine muscle damage. In the tourniquet group the pH fell to 6.35 after three hours, and there was no evidence of irreversible muscle damage on biopsy histology. But in the compartment syndrome group, the pH fell to 5.88 and biopsy revealed evidence of irreversible muscle damage. Although the pH at which irreversible structural damage to human skeletal muscle cells occurs has not been determined, extremely low muscle pH may be responsible for the complications described following exceptionally prolonged tourniquet times. In addition, there may be an additive effect through direct crush of the tissues immediately under the tourniquet in addition to tourniquet-induced ischaemia.

In conclusion, using adapted pH monitoring equipment, our preliminary study demonstrates that it is possible to directly measure skeletal muscle pH over time in human subjects. We can also conclude that the application of a tourniquet results in a fall in skeletal muscle pH over time in patients undergoing short arthroscopic procedures. Further work is required to measure pH decline during longer procedures with a tourniquet, and to correlate falls in pH with clinical symptoms. However, in future it may be useful to use pH monitoring to detect and therefore prevent extreme ischaemia during much longer cases, particularly in susceptible individuals e.g. patients with arterial disease, whereby pH readings may indicate when the safe period of tourniquet release is close to being exceeded.

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Author information:

- D. R. W. MacDonald, MRCS, Speciality Registrar, Orthopaedics
- D. W. Neilly, FRCS, Speciality Registrar, Orthopaedics
- A. J. Johnstone, FRCS, Consultant Orthopaedic Surgeon
Aberdeen Royal Infirmary, Aberdeen, UK.
- K. E. Elliott, FRCS, Consultant Orthopaedic Surgeon, University Hospital
Southampton, Southampton, UK.

Author contributions:

- D. R. W. MacDonald: Conducted the formal analysis, Drafted the manuscript.
- D. W. Neilly: Conducted the formal analysis, Reviewed and edited the manuscript.
- K. E. Elliott: Collected and curated the data, Carried out the project administration, Conducted the formal analysis.
- A. J. Johnstone: Conceptualized, visualized, and supervised the study, Handled the methodology, Reviewed and edited the manuscript.

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- Ethical approval was obtained from our local research ethics committee.

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