

# Does the method of expression of venous blood affect ischaemia/reperfusion damage in tourniquet use? An experimental study on rabbits

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## ABSTRACT

The aim of this study was to compare the ischaemia and reperfusion phases of two tourniquet application models (Group 1: expressing the blood by a sterile rubber bandage and Group 2: elevation of the limb for several minutes) using an analysis of ischaemia/reperfusion parameters and blood pH.

Sixteen New Zealand rabbits were used. Muscle samples were extracted from the triceps surae; at phase A (baseline: just before tourniquet application), phase B (ischaemia: 3 h after tourniquet inflation) and phase C (2 h after tourniquet deflation). Nitrite, nitrate, reduced glutathione, myeloperoxidase, malondyaldehyde were measured in the samples. Blood pH was also measured at each phase.

Group 2 had significantly decreased nitrite ( $p = 0.007$ ) and nitrate ( $p = 0.01$ ) levels compared to Group 1 while passing from phase A to phase B. The pH decrease through the phases was significant within Group 1 ( $p = 0.006$ ) and was not significant within Group 2 ( $p = 0.052$ ).

Lower levels of NO metabolites nitrate and nitrite, result from tourniquet use with incomplete venous blood expression by elevation. Also, with this technique severe acidosis is less likely to occur than when a tourniquet is used with expression of the venous blood by rubber bandage. These findings may help in the decision of which tourniquet technique is to be used for potentially long operations which may exceed 2 h.

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## Introduction

Pneumatic tourniquets have been widely used since first described by Harvey Cushing in 1904.<sup>1</sup> The use of such a tourniquet provides a bloodless environment during surgery, helps in the precise identification of anatomical structures, decreases the need for transfusion and shortens operating time.<sup>2,3</sup> On the other hand, both high tissue pressure and ischaemia cause cell destruction, while the increased free oxygen radicals caused by reperfusion after deflation of the tourniquet, trigger local and remote organ damage.<sup>2,4</sup>

The damage caused by ischaemia/reperfusion is a type of inflammation based on neutrophil activation. Although reperfusion is crucial to prevent further ischaemia, reperfusion itself causes damage as the result of a cascade characterised by excessive neutrophil migration, destruction of the microvascular barrier and

oedema.<sup>5</sup> The main event in reperfusion injury is the adhesion of leukocytes to endothelia passing from post-capillary venules. Accumulation of leukocytes in tissues causes parenchymal damage by inducing the liberation of various oxidants, enzymes and cytokines. Life-threatening complications such as an increase in plasma viscosity,<sup>6</sup> alterations in the coagulation system, increase in fibrinolysis,<sup>4</sup> cardiovascular system disorders,<sup>4</sup> pulmonary complications, arrhythmias,<sup>7</sup> metabolic asidosis,<sup>8</sup> and neuromuscular damage<sup>9</sup> have all been linked with ischaemia/reperfusion damage.

Although ischaemia/reperfusion injury has been widely investigated from various aspects, there is no study comparing the two techniques, which are widely used before inflation of the tourniquet; elevation of the limb for several minutes or expressing the blood by a sterile rubber bandage. Our hypothesis was that not expressing the blood completely might have some protective role against ischaemia rather than total expression of the blood from the extremity, as tissues might still supply some of their requisite from the residual blood in the limb. The aim of this study was to compare the ischaemia and reperfusion phases of two tourniquet application models using an analysis of nitrite, nitrate, reduced glutathione, myeloperoxidase, malondyaldehyde and blood pH.

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**Table 1**  
Study groups and their characteristics.

|         | Expression of venous blood with rubber bandage | Tourniquet application | Ischaemic period (h) | Reperfusion period (h) | n |
|---------|------------------------------------------------|------------------------|----------------------|------------------------|---|
| Group 1 | +                                              | +                      | 3                    | 2                      | 8 |
| Group 2 | –                                              | +                      | 3                    | 2                      | 8 |

## Materials and methods

Approval for the study was obtained from the IRB and the Local Animal Ethics Committee with reference number 2309. The study was conducted at the Experimental and Surgical Animal Research Laboratory in a manner similar to the acute ischaemia/reperfusion model described by Hardy et al.<sup>8</sup>. Sixteen New Zealand rabbits were used with a mean weight of 3.4 kg (range: 2.7–4.3 kg). “Guide for the Care and Use of Laboratory Animals” standards were applied during the study.<sup>10</sup>

Anaesthesia was administered at room temperature as intramuscular ketamine HCl (Ketalar) injected in the left forefoot at a dose of 30 mg/kg. Blood pressure and pulse were monitored in the left forefoot. Arterial and venous lines were inserted through the ear in all test subjects to screen blood gases during sampling and for isotonic saline infusion respectively. All rabbits were given oxygen support at a rate of 3 l/min by mask. Haemodynamic parameters of blood pressure and heart rate were recorded via their left forefoot at 30 min intervals during the procedure. Body temperature was monitored rectally and 38° was maintained. A total of 70 ml of isotonic saline was infused; one third was given at anaesthesia for 30 min until the tourniquet was inflated, one third during ischaemia (3 h after tourniquet inflation), and the remaining third during reperfusion (2 h after tourniquet deflation).

The rabbits were randomly allocated to two groups of eight. In Group 1 the blood was completely expressed by a rubber bandage from the extremity. In Group 2 the blood was partially expressed by elevating the limb for 2 min (Table 1).

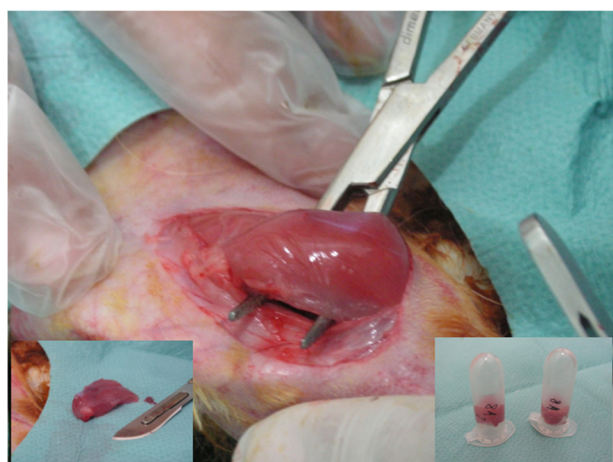
In both groups, before the tourniquet was inflated, muscle biopsy sampling was performed at the triceps surae muscle of the lower extremity of the left hind leg (sampling A) (Fig. 1). Double samples were taken against the possibility of having insufficient material for samples. Each sample size was 1 cc. Then, an Esmarch bandage was applied as a tourniquet to the right hind leg of the rabbits at the level of the hip joint in order to arrest arterial circulation, after expressing the blood from the extremity by a rubber bandage in Group 1 (Fig. 2A) and with only elevating the

extremity for 2 min without expressing by a rubber bandage in Group 2 (Fig. 2B). After applying the tourniquet one nail of each rabbit was extracted and the absence of bleeding was confirmation of the tourniquet functioning (Fig. 2C).

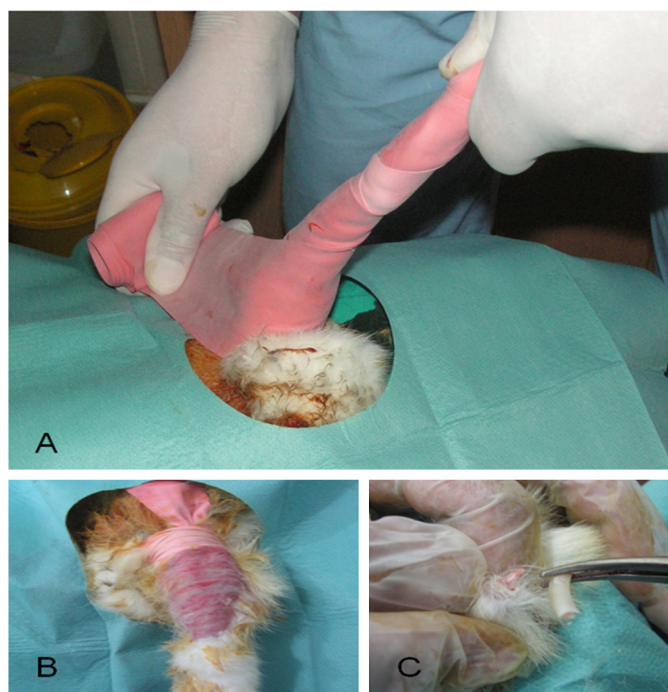
At the end of 3 h of ischaemia and before the tourniquet was deflated, ischaemic muscle biopsy sampling was performed at the triceps surae muscle (sampling B). After the tourniquet was deflated, a biopsy was performed in the same muscle group following 2 h of reperfusion (sampling C) and sent to the biochemistry laboratory for evaluation of pH, nitrite, nitrate, GSH, MPO and MDA levels. Samples B and C were taken at least 5 mm away from the previous biopsy sites to leave tissues intact. In all test subjects, arterial blood gases were measured during A–C muscle biopsy sampling (A–C arterial blood gases) and their results were recorded.

Tissue samples were immediately frozen and stored at –80 °C until nitric oxide metabolites, MDA, MPO activity and GSH were measured. On the working day, samples were homogenised in buffer on ice and assayed for nitric oxide metabolites, MDA, MPO and GSH contents using the methods described.

GSH was measured by the colorimetric method described by Elman.<sup>11</sup> Metaphosphoric acid (0.5 mol l<sup>–1</sup>; W/V 1/5) was added to the homogenate and the mixture was centrifuged at 3500 rpm. Three millilitres of supernatant was treated with 2.0 ml of phosphate buffer (0.1 mol l<sup>–1</sup>, pH 8.0), 5 ml distilled water, and 0.02 ml of DTNB reagent (39.6 ml of 5,5'-dithiobisnitro benzoic acid in 10 ml 0.1 mol l<sup>–1</sup> pH 7 phosphate buffer). The absorbance was read at 412 nm, using 80, 90, 100, and 125 mmol l<sup>–1</sup> reduced



**Fig. 1.** Biopsy taken from the triceps surae muscle.



**Fig. 2.** (A) Venous blood was completely expressed by Esmarch bandage in Group 1. (B) Tourniquet application to high thigh. (C) Checking the tourniquet by nail extraction.

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