Compartment syndrome-induced muscle injury is diminished by the neutralization of pro-inflammatory cytokines

Erin Donohoe, MBBS, MBChB\textsuperscript{a,b}, Aurelia Bihari, PhD\textsuperscript{a,b}, Emil Schemitsch, MD, FRCSC\textsuperscript{a}, David Sanders, MD, FRCSC\textsuperscript{a}, Abdel-Rahman Lawendy, MD, PhD, FRCSC\textsuperscript{a,b}

\textsuperscript{a}Division of Orthopaedic Surgery, University of Western Ontario, \textsuperscript{b}Centre for Critical Illness Research, Lawson Health Research Institute, London, Ontario, Canada

Abstract

\textbf{Objectives}: Compartment syndrome (CS) is one of the most devastating consequences of musculoskeletal trauma. The pathophysiology of CS includes elevation of intracompartmental pressure (ICP), causing damage to the microcirculation, decreased oxygen delivery, tissue anoxia, and cell death. CS is a combined ischemic and inflammatory condition that induces the systemic inflammatory cascade. In complete ischemia, within the first hour of reperfusion, a peak in the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-\( \alpha \)) has been previously reported. The purpose of this study was to examine the suspected systemic inflammatory cytokine/chemokine release in response to CS, and to evaluate the microvascular dysfunction, tissue injury, and inflammatory response following the neutralization of pro-inflammatory cytokines TNF-\( \alpha \) and/or interleukin-1 beta (IL-1\( \beta \)).

\textbf{Methods}: Twenty-eight male Wistar rats were randomly assigned into 5 groups: Sham (no CS), CS (with isotype control), CS+TNF-\( \alpha \) neutralizing antibody (NA), CS+IL-1\( \beta \) NA, CS+Combo (both TNF-\( \alpha \) and IL-1\( \beta \) NA). CS was induced by elevation of ICP above 30 mm Hg through an infusion of isotonic saline into the anterior compartment of the hind limb for 2 hours; NA were administered just prior to fasciotomy. Microvascular perfusion, cellular tissue injury, and inflammatory response within the extensor digitorum longus muscle were assessed using intravital video microscopy for 45 minutes after fasciotomy. Systemic levels of 24 different cytokines/chemokines were also measured, using the xMAP Luminex technology.

\textbf{Results}: Of the 24 cytokines/chemokines sampled, 6 were significantly elevated from their baseline levels, and included the pro-inflammatory cytokines TNF-\( \alpha \), IL-1\( \beta \), growth-related oncogene/keratinocyte chemotactant (GRO/KC), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1\( \alpha \)), and the anti-inflammatory cytokine IL-10. CS resulted in a significant decrease in microvascular perfusion, from 75\( \pm \)2% continuously perfused capillaries in the sham to 31\( \pm \)4% in CS (\( P < .001 \)), a significant increase in tissue injury (0.33\( \pm \)0.4 versus 0.04\( \pm \)0.01 in sham) and leukocyte activation (14\( \pm \)2 adherent leukocytes/1000\( \mu \text{m} \)^2 versus 2\( \pm \)1 adherent leukocytes/100\( \mu \text{m} \)^2 in sham, \( P < .001 \)). CS-associated tissue injury was significantly decreased with TNF-\( \alpha \) neutralization (\( P < .05 \)), both when administered alone or in combination with IL-1\( \beta \) (\( P < .05 \)). Additionally, TNF-\( \alpha \) neutralization blocked CS-associated leukocyte activation (\( P < .05 \)); IL-1\( \beta \) neutralization also diminished leukocyte adhesion (\( P < .05 \)). Perfusion remained virtually unchanged in CS animals treated with NA (36\( \pm \)4%, 32\( \pm \)3% and 30\( \pm \)2% in CS+TNF-\( \alpha \), CS+IL-1\( \beta \) and CS+Combo groups, respectively).

\textbf{Conclusion}: The results of this study indicate that CS induces a systemic inflammatory response, as evidenced by upregulation of inflammatory cytokines/chemokines in circulation. Neutralization of TNF-\( \alpha \) led to a significant reduction in tissue injury; however, it had no effect on the CS-induced microvascular dysfunction. This suggests a distinct role of TNF-\( \alpha \) in the pathophysiology of muscle injury in CS.

\textbf{Abbreviations}: BB = bisbenzimide, OPC = continuously-perfused capillaries, CS = compartment syndrome, EB = ethidium bromide, EDL = extensor digitorum longus, ELISA = enzyme-linked immunosorbent assay, GRO/KC = growth-related oncogene/keratinocyte chemotactant, I/R = ischemia-reperfusion, ICP = intracompartmental pressure, IL-1\( \beta \) = interleukin-1 beta, IPC = intermittently-perfused capillaries, IVM = intravital video microscopy, MCP-1 = monocyte chemoattractant protein 1, MIP-1\( \alpha \) = macrophage inflammatory protein 1 alpha, NA = neutralizing antibodies, NPC = non-perfused capillaries, ROS = reactive oxygen species, TNF-\( \alpha \) = tumor necrosis factor alpha.

\textbf{Keywords}: compartment syndrome, cytokines/chemokines, inflammation, tissue injury, tissue perfusion, TNF-\( \alpha \), IL-1\( \beta \), cytokine neutralization

The study was supported by 2015–2016 Orthopaedic Trauma Association Resident Research grant, awarded to Dr Donohoe, and Lawson Health Research Institute Internal Research Fund (IRF-19-14), awarded to Dr Lawendy.

This work was presented at the 2016 Orthopaedic Trauma Association Annual Meeting in Washington, DC.

The authors have no conflicts of interest to disclose.

\textsuperscript{a} Division of Orthopaedic Surgery, University of Western Ontario; \textsuperscript{b}Centre for Critical Illness Research, Lawson Health Research Institute, London, Ontario, Canada

\textsuperscript{c}Corresponding author. Address: Rm A6-152, Victoria Research Labs, London Health Sciences Centre (Victoria Hospital), 800 Commissioners Road, East, London, Ontario N6A 4G5, Canada. Tel: +1 519 685 8300; fax: +1 519 685 8341. E-mail address: relka.bihari@lhsc.on.ca (A. Bihari).

Copyright © 2018 The Authors. Published by Wolters Kluwer Health, Inc. on behalf of the Orthopaedic Trauma Association. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

OTA (2018) e011

Received: 13 February 2018 / Received in final form: 21 August 2018 / Accepted: 28 August 2018

http://dx.doi.org/10.1097/OB.0000000000000011
Introduction

CS remains one of the most devastating and challenging sequelae of musculoskeletal trauma. CS develops secondary to increased pressure within a closed osseofascial compartment, most commonly following a tibial diaphyseal fracture. Elevated ICP compromises the microcirculation, leading to decreased oxygen and nutrient delivery, tissue anoxia and, ultimately, myonecrosis. The pathophysiology of CS-associated tissue injury is only partially understood. While ischemia-reperfusion (I/R) appears to be the main driving force, there is a critical distinction between CS and complete I/R: unlike complete I/R, tissue necrosis and ischemic injury in CS ensue despite a patent macrocirculatory system and palpable distal pulses. Previous studies have demonstrated that CS-induced tissue injury is more extensive than that of a complete ischemic insult of the same duration.

Normal microvascular perfusion is comprised of predominantly continuously perfused capillaries (CPC). Elevated ICP results in a shift of perfusion towards IPC and NPC, leading to a “low flow” ischemic state. As a result, the metabolic demands of the tissue cannot be met, resulting in the production of reactive oxygen species (ROS). ROS, generated following the reintroduction of oxygen into previously ischemic tissue are known to trigger the acute inflammatory cascade and eventual cellular death via apoptosis or necroptosis. Unlike a complete I/R (as observed following organ transplantation and re-vascularization procedures), the defined phases of injury (ischemia, reperfusion) cannot be clearly delineated in a “low-flow” ischemia, initiating an early and ongoing reperfusion injury concurrent with ischemic period. The reperfusion injury not only persists throughout the duration of the CS, but also it is further intensified by the restoration of normal blood flow into the capillary bed after fasciotomy.

Activated leukocytes appear to play a major role in CS pathophysiology. While leukopenia does not restore or maintain perfusion, it appears to provide significant protection against muscle injury. The pro-inflammatory cytokine TNF-α has been shown to be acutely upregulated following reperfusion in multiple I/R studies.

The purpose of this study was to examine the specific cytokines/chemokines released during CS in a rat model, as well as to evaluate the microvascular dysfunction, tissue injury, and leukocyte activation following the neutralization of pro-inflammatory cytokines TNF-α and IL-1β at the time of fasciotomy. We hypothesized that CS is associated with the initiation of the systemic inflammatory cascade, and that neutralization of ischemia-reperfusion injury-linked cytokines would diminish the microvascular dysfunction and tissue injury associated with CS.

Materials and methods

Animal preparation

The experimental protocol was approved by the Canadian Council on Animal Care and the Animal Use Subcommittee at The University of Western Ontario. Male Wistar rats (body weight 180–250g) were used for all experiments.

Animals were anesthetized with isoflurane (5% induction, 2% maintenance) in a 1:1 O₂:N₂ mixture. The left carotid artery was cannulated for the continuous monitoring of blood pressure, fluid replacement, and blood sampling; core body temperature was maintained at 37°C by the means of a heat lamp. A normotensive and normothermic model of CS was used.

CS was induced by an infusion of isotonic saline into the anterior compartment of the right-hind limb via a 24-gauge angiocatheter to raise the ICP, as previously described. Compartment pressures were measured by an electronic compartmental pressure monitoring system (Synthes USA, Paoli, Pennsylvania), inserted into the posterior compartment via a 14-gauge angiocatheter. Elevated ICP was maintained at 30 to 40 mm Hg for 2 hours, followed by fasciotomy and 45 minutes of reperfusion. The skeletal muscle was then imaged by intravital video microscopy (IVVM).

Experimental groups

Animals were randomly assigned into 1 of 5 groups: Sham (n = 8), CS (with isotype control) (n = 8), CS with TNF-α neutralization (CS + TNF-α) (n = 4), CS with IL-1β neutralization (CS + IL-1β) (n = 4), and CS with neutralization of both TNF-α and IL-1β (CS + Combo) (n = 4). Sham animals underwent all the procedures as the CS groups, but they did not receive saline infusion into the anterior compartment of the hind limb and the ICP was maintained at the baseline level of 0 mm Hg.

Serum cytokines/chemokines

All blood samples were drawn from the indwelling carotid line at 7 different time points: baseline (just prior to the elevation of ICP); at 1 hour CS; at 2 hours CS, just prior to fasciotomy; at 10 minutes reperfusion, at 20 minutes reperfusion, at 30 minutes reperfusion; at 45 minutes reperfusion, just prior to IVVM. At each time point, 0.1 mL of blood was withdrawn and replaced with 0.1 mL of normal saline.

Serum levels of cytokines/chemokines were assessed by xMAP Luminex assay (EMD Millipore, Mississauga, Ontario), as per manufacturer’s instructions, simultaneously testing 24 different cytokines/chemokines: TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17, IL-18, IFN-γ, Eotaxin, IP-10, MIP-1α, Leptin, MCP-1, GRO/KC, RANTES, GM-CSF, G-CSF, and VEGF. The samples were analyzed using Luminex 200 instrument (EMD Millipore, Mississauga, Ontario).

Neutralization of serum TNF-α and IL-1β

NA to TNF-α and/or IL-1β (Ebioscience, San Diego, CA) was administered to CS animals just before fasciotomy at a dose of 3.5 × 10^4 neutralizing units/100g body weight (0.3 mL IV). Syrian hamster IgG (Ebioscience, San Diego, California) was used as an isotype control. Neutralization of serum TNF-α and IL-1β was confirmed by enzyme-linked immunosorbent assay (ELISA, Pierce Biotechnology, c/o Thermo Scientific, Rockford, Illinois) as per manufacturer’s instructions. ELISAs were sensitive to <5 pg/mL.

Intravital video microscopy (IVVM)

Following fasciotomy, the extensor digitorum longus (EDL) muscle was prepared for IVVM, as previously described. Briefly, following isolation of EDL, the animal was transferred onto the stage of an inverted microscope (Nikon, Nikon Instruments, Inc., Mississauga, Ontario, Canada); the EDL muscle was detached from its bony insertion and reflected into a saline bath containing 5 µg/mL of each of the fluorescent vital dyes bisbenzimide (BB; excitation wavelength (Ex) 343 nm, emission wavelength (Em) 390 nm) and Oregon Green 488 BAPTA-1 AM (OG; Ex 488 nm, Em 510 nm) to image Ca²⁺ levels.
Serum levels of cytokines/chemokines detected in a rat model of CS.

| Cytokine/Chemokine | Baseline, pg/mL | At 2-hour CS, pg/mL | Fold change | P-value  |
|--------------------|----------------|---------------------|-------------|----------|
| TNF-α              | 18.1 ± 3.5      | 1495.1 ±152.7 †      | 82          | <.0004   |
| IL-1β              | 40.0 ±20.0      | 141.3 ±18.6 †        | 3           | .0384    |
| FN-γ               | 326.6 ±98.6     | 360.4 ±159.3     | 0.1         | .8463    |
| IL-6               | 2566 ±1758      | 2351 ±1494         | –0.1        | .9084    |
| MIP-1α             | 0.0 ±0.0        | 84.8 ±41.0 †       | 8           | .0077    |
| MIP-1              | 313.4 ±31.4     | 698.8 ±30.6 †      | 1           | .0172    |
| GRO/KC             | 990.2 ±213.3    | 2984 ±1310 †       | 2           | .0001    |
| L-1α               | 473.9 ±291.7    | 434.7 ±179.3       | –0.1        | .8887    |
| Leptin             | 9906 ±1038      | 8066 ±1353         | –0.1        | .5344    |
| Eotaxin            | 228.0 ±161.3    | 175.7 ±144.3       | –0.2        | .4431    |
| IL-13              | 225.3 ±60.0     | 254.9 ±106.9       | 0.1         | .7161    |
| IL-12p70           | 196.1 ±88.1     | 199.2 ±83.5        | 0           | .9718    |
| IL-18              | 86.6 ±7.3       | 76.4 ±6.1          | –0.1        | .1621    |
| P-10               | 145.4 ±108.6    | 155.7 ±108.9       | 0.1         | .9424    |
| RANTES             | 1673 ±250       | 1682 ±193          | 0           | .3560    |
| IL-10              | 68.4 ±68.4      | 1138.3 ±372.3 †    | 16          | .0012    |

Twenty-four different cytokines and chemokines were tested; those with detectable values are shown here.

\*P < .05.
and 12.3 ± 7.1 pg/mL, respectively. Levels of TNF-α at 2 hours and 45 minutes reperfusion time-matched points were 14.8 ± 3.8 pg/mL, and 12.6 ± 5.9 pg/mL, respectively, while those of IL-1β were 11.0 ± 6.0 pg/mL and 14.0 ± 8.0 pg/mL, respectively (Fig. 1).

Microvascular perfusion
Elevation of ICP resulted in significant changes to microvascular perfusion, as shown in Figure 2. The number of CPC decreased from 75 ± 2% in the sham to 31 ± 3%, 36 ± 4%, 31 ± 3%, and
30 ± 2% in the CS, TNF-α, IL-1β and Combo neutralizing groups, respectively (P < .001). There were no significant differences in CPC between the CS and any of the neutralizing groups.

The number of IPC increased from 11 ± 2% in sham to 19 ± 1%, 25 ± 3%, 36 ± 4% and 38 ± 2% in the CS and TNF-α, IL-1β and Combo neutralizing groups, respectively (P < .001). The number of IPC in all neutralizing groups was significantly higher than that in CS group (P < .05).

The number of NPC increased from 14 ± 1% in sham to 50 ± 3%, 39 ± 3% 33 ± 2% and 32 ± 2% in the CS and TNF-α, IL-1β and Combo neutralizing groups, respectively (P < .001). The number of NPC in the CS+TNF-α neutralizing group was significantly lower as compared to the CS group (P < .05).

**Tissue injury**

Elevation of ICP resulted in a significant increase in tissue injury, from 0.04 ± 0.02 in sham to 0.33 ± 0.04 in CS group (P < .001) (Fig. 3). Administration of TNF-α NA resulted in a decrease to 0.19 ± 0.04 (P < .001) and 0.13 ± 0.02 (P < .001) in CS+TNF-α and CS + Combo groups, respectively, while neutralization of IL-1β had no effect (0.29 ± 0.02 in CS+IL-1β group, not significant).

**Inflammation**

Elevation of ICP resulted in an increase in leukocyte rolling, from 2 ± 1 leukocytes/30 s/1000 μm² in sham to 10 ± 3 leukocytes/30 s/1000 μm² in CS group (P < .001). Administration of TNF-α NA resulted in a significant decrease to 2 ± 1 and 3 ± 1 rolling leukocytes/30 s/1000 μm² in CS+TNF-α and CS+Combo groups, respectively (P < .01) (Fig. 4A). Administration of IL-1β following CS had no effect (15 ± 3 rolling leukocytes/30 s/1000 μm², not significant).

There was a significant increase in leukocyte adherence following elevation of ICP, from 2 ± 1 to 14 ± 2 leukocytes/30 s/1000 μm² in CS group (P < .001) (Fig. 4B). Administration of TNF-α NA produced a significant decrease to 1 ± 0 adherent leukocytes/30 s/1000 μm² in both CS+TNF-α and CS+Combo groups (P < .001). Administration of IL-1β also significantly decreased leukocyte adherence (6 ± 1 adherent leukocytes/30 s/1000 μm², P < .05).

**Discussion**

In spite of the fact that CS has been a recognized complication of musculoskeletal trauma since Richard von Volkman’s description of ischemic contracture in 1881, the pathophysiology of CS remains largely unknown. The use of IVVM imaging has been instrumental in illustrating the derangement of microvascular perfusion following a CS insult. Under live conditions, we were able to observe the presence of CPC, IPC, and NPC within the same capillary bed, validating CS as a “low-flow” ischemic environment.

Inflammation has been shown to be a major contributor to the microvascular dysfunction associated with I/R. (TNF-α has been
shown to be elevated following a complete I/R injury\[19,20\], and hence CS. The purpose of this study was to identify the mediators (i.e., cytokines/chemokines) involved, and to observe the effect of neutralizing 2 major pro-inflammatory cytokines, TNF-α, and IL-1β, on microvascular perfusion, tissue injury, and leukocyte activation in an animal model of CS.

This study confirms that CS induces a systemic response, as evidenced by the upregulation of pro-inflammatory cytokines/chemokines in circulation. Of the 14 cytokines/chemokines detected in the serum of rats challenged with CS, 6 were significantly elevated from their baseline levels after 2 hours of ICP elevation (\(P<.05\)): TNF-α, IL-1β, GRO/KC, MCP-1, MIP-1α, and IL-10. With the exception of IL-10, (which is an anti-inflammatory cytokine),\[26\] all detected cytokines/chemokines are pro-inflammatory.\[27–35\]

TNF-α is a powerful pro-inflammatory cytokine released following trauma, inflammation, or infection.\[36\] TNF-α acts as a chemoattractant for neutrophils, causes up-regulation of cytokine/chemokine production, and activates cell death via apoptosis and necroptosis.\[27–30,37\] Similarly, IL-1β is another pro-inflammatory cytokine that plays an important role in sterile inflammation.\[28\] IL-1β is produced by activated macrophages, monocytes, endothelial cells, fibroblasts, and appears to be involved in cell proliferation, differentiation and apoptosis, mediating an inflammatory sequence similar to that of TNF-α.\[28\] As both TNF-α and IL-1β have been demonstrated to play a significant role in I/R,\[29,38\] our current study focused on assessing the contribution of these 2 cytokines to the pathophysiology of CS.

CS resulted in a significant rise in systemic TNF-α and IL-1β, in conjunction with a marked increase in activated leukocytes. Modulating the inflammatory response significantly decreased leukocyte activation, illustrated by a decrease in both rolling and adherent leukocytes within the post-capillary venules (\(P<.05\) and \(P<.0001\), respectively).

Tempering the inflammatory response through neutralization of TNF-α (alone or in combination with IL-1β) also led to a significant relative reduction of approximately 40% in tissue injury following 2 hours of elevated ICP. Neutralization of IL-1β by itself had no appreciable effect; however, when given in combination with TNF-α, it augmented the reduction in parenchymal injury to levels previously found in a leukopenic model of CS.\[14\] One possible explanation as to why neutralization of IL-1β alone failed to offer any protection may be due to the existence of temporal relationship between TNF-α and IL-1β release.\[38\] In I/R injury, TNF-α release appears to precede that of

---

**Figure 3.** The effect of pro-inflammatory cytokine neutralization on skeletal muscle injury in a rat model of CS. Administration of TNF-α or a combination of TNF-α/IL-1β NA at fasciotomy significantly diminished CS-induced tissue injury; neutralization of IL-1β alone had no effect (\(*P<.001\) from sham, †\(P<.01\) from CS; one-way ANOVA).
IL-1β; this would indicate that IL-1β probably plays a more significant role at later stages of ischemia. Thus, our findings suggest that, although TNF-α appears to play a significant role in the inflammatory response associated with CS, the contribution of other cytokines/chemokines (e.g., IL-1β) to the pathophysiology is not inconsequential.

Neutralizing TNF-α and/or IL-1β at the time of fasciotomy did not provide any protection to the microvascular perfusion following a CS insult. These results corroborate previous work by our group, whereby leukopenia was not protective in preventing the microvascular dysfunction associated with CS.\(^{11,14}\) Again, a distinction from complete I/R must be highlighted: following complete ischemia, microvascular dysfunction, and tissue injury were significantly decreased in a leukopenic I/R animal model.\(^{17}\) Our current study, therefore, provides further evidence that the pathologic changes in microvascular dysfunction following CS have a distinct pathophysiology compared to complete I/R injury.

This study demonstrates that CS is associated with a significant activation of the systemic inflammatory response, and its initiation should, therefore, be considered in the pathophysiology of CS. In addition to its role as an acute inflammatory mediator, TNF-α has also been implicated in initiating cell death via apoptosis and necroptosis.\(^{39}\) The necroptotic pathway is also associated with the tissue injury following I/R.\(^{10,11}\) In contrast to apoptosis, necroptosis generates a systemic inflammatory response. It can be inferred from our findings that this pathway of programmed cell death may contribute to the tissue injury associated with CS. It is plausible that the early and sustained reperfusion injury associated with ’low-flow’ ischemia induces an early and sustained stimulus for the necroptotic pathway. This could in part, explain the greater tissue injury associated with CS versus complete I/R.

Surprisingly, neutralization of TNF-α (alone or in combination with IL-1β) did not confer the same type of protection to the microvasculature as that seen following administration of carbon monoxide (CO) donor, CORM-3, in a rat model of CS.\(^{32}\) CO has been shown to exhibit potent vasodilatory, anti-inflammatory and anti-apoptotic properties in various models of disease.\(^{13,44}\) As such, the results of our current study confirm that inflammatory response is not the only component of CS pathophysiology, although it plays a major role. Hence, neutralization of pro-inflammatory cytokines does not address other factors that contribute to CS-associated damage (e.g., vasodilation, ROS production, apoptosis) and thus is insufficient in restoring the microcirculation back to normal.

To our knowledge, this is the first study that directly confirms CS as an inflammatory process. The systemic inflammatory cascade is complex and redundant, and these results are merely the beginning steps in an attempt to extrapolate the pathophysiologic mechanism by which CS injury occurs.

References

1. Matsen FA3rd. Compartmental syndrome. An unified concept. Clin Orthop Relat Res. 1975;113:8–14.
2. Whitesides TE, Haney TC, Morimoto K, et al. Tissue pressure measurements as a determinant for the need of fasciotomy. Clin Orthop Relat Res. 1975;113:43–51.
3. Mubarak SJ, Owen CA, Hargens AR, et al. Acute compartment syndromes: diagnosis and treatment with the aid of the wick catheter. J Bone Joint Surg Am. 1978;60:291.
4. Rorabeck CH, Clarke KM. The pathophysiology of the anterior tibial compartment syndrome: an experimental investigation. J Trauma. 1978;18:299–304.
5. Matsen FA3rd, Winsquist RA, Krugmire RBJr. Diagnosis and management of compartmental syndromes. J Bone Joint Surg Am. 1980;62:286-291.
6. Rorabeck CH. The treatment of compartment syndromes of the leg. J Bone Joint Surg Br. 1984;66:93–97.
7. Tornetta P3rd, Templeman D. Compartment syndrome associated with tibial fracture. Instr Course Lect. 1997;46:303–308.
8. McQueen MM, Gaston P, Court-Brown CM. Acute compartment syndrome. Who is at risk? J Bone Joint Surg Br. 2000;82:200–203.
9. Sheridan GW, Matsen FA. An animal model of the compartmental syndrome. Clin Orthop Relat Res. 1975;113:36–42.
10. Harvey E, Sanders D, Shuler M, et al. What’s new in acute compartment syndrome? J Orthop Trauma. 2012;26:699–702.
11. Seddon HJ. Volkman’s ischaemia in the lower limb. J Bone Joint Surg Br. 1966;48:627–636.
12. Heppenstall RB, Scott R, Saagea A, et al. A comparative study of the tolerance of skeletal muscle to ischemia. Tourniquet application
13. Lawendy AR, Sanders DW, Bihari A, et al. Contribution of microvascular dysfunction to cellular injury in compartment syndrome: an experimental rodent model. Can J Surg. 2011;54:149–200.

14. Lawendy A, Bihari A, Sanders D, et al. Contribution of inflammation to cellular injury in compartment syndrome: an experimental rodent model. Bone Joint J. 2015;97-B:539–543.

15. Gute DC, Ishida T, Yarimizu K, et al. Inflammation and ischemia in rat muscle ischaemia–reperfusion injury. Ann Plast Surg. 2003;54:313–317.

16. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol. 2001;280:C719–C741.

17. Forbes TL, Harris KA, Jamieson WG, et al. Leukocyte activity and tissue injury following ischemia–reperfusion in skeletal muscle. Microvasc Res. 1996;51:275–287.

18. Harris AG, Skalak TC. Effects of leukocyte capillary plugging in skeletal muscle ischemia-reperfusion injury. Am J Physiol. 1996;271:H2633–H2640.

19. Gary MG, Guice KS, Oldham KT, et al. Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. Ann Surg. 1990;212:694–700.

20. Brock RW, Lawlor DK, Harris KA, et al. Initiation of remote hepatic injury in the rat: interactions between Kupffer cells, tumor necrosis factor-alpha, and microvascular perfusion. Hepatology. 1999;30:137–142.

21. Krishnadasan B, Naidu BV, Byrne K, et al. The role of proinflammatory cytokines in lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg. 2003;125:261–272.

22. Potter RF, Dietrich HH, Tyml K, et al. Ischemia-reperfusion induced microvascular dysfunction in skeletal muscle: application of intravital video microscopy. Int J Microcirc Clin Exp. 1993;13:173–186.

23. Forbes TL, Carson M, Harris KA, et al. Skeletal muscle injury induced by ischemia-reperfusion. Can J Surg. 1995;38:56–63.

24. Manjoo A, Sanders D, Lawendy A, et al. Indomethacin reduces cell damage: shedding new light on compartment syndrome. J Orthop Trauma. 2010;24:526–529.

25. von Volkmann R. Die ischämischen muskellähmungen und kontrakturen. Zentralbl Chir. 1881;8:801–803.

26. Mosser D, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunol Rev. 2008;226:205–218.

27. Ascenzi Gennaro, M, Cupo S, et al. Cytokines play a role in skeletal muscle ischemia and reperfusion? J Cardiovasc Surg (Torino). 1992;33:588–592.

28. Yi ES, Ulich TR. Endotoxin, interleukin-1, and tumor necrosis factor cause neutrophil-dependent microvascular leakage in postcapillary venules. Am J Pathol. 1992;140:659–663.

29. Seekamp A, Warren JS, Remick DG, et al. Requirements for tumor necrosis factor-alpha and interleukin-1 in limb ischemia/reperfusion injury and associated lung injury. Am J Pathol. 1993;143:453–463.

30. Zhang F, Hu EC, Gerzenstein J, et al. The expression of proinflammatory cytokines in the rat muscle flap with ischemia-reperfusion injury. Ann Plast Surg. 2003;54:313–317.

31. Gao D, Madi M, Ding C, et al. Interleukin-1β mediates macrophage-induced impairment of insulin signaling in human primary adipocytes. Am J Physiol Endocrinol Metab. 2014;307:E289–E304.

32. Bechara G, Chai H, Lin P, et al. Growth related oncogene-alpha (GRO-a): roles in atherosclerosis, angiogenesis and other inflammatory conditions. Med Sci Monit. 2007;13:RA87–90.

33. Shireman PK, Contreras-Shannon V, Ochoa O, et al. MCP-1 deficiency causes altered inflammation with impaired skeletal muscle regeneration. J Leukoc Biol. 2007;81:775–785.

34. Yadav A, Saini V, Arora S. MCP-1: chemotaxin with a role beyond immunity: a review. Clin Chim Acta. 2010;411:1570–1579.

35. Cook D. The role of MIP-1α in inflammation and hematopoiesis. J Leukoc Biol. 1996;59:61–66.

36. Stein M, Gordon S. Regulation of tumor necrosis factor (TNF) release by murine peritoneal macrophages: role of cell stimulation and specific phagocytic plasma membrane receptors. Eur J Immunol. 1991;21:431–437.

37. Parameswaran N, Patial S. Tumor necrosis factor—α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20:87–103.

38. Lawlor DK, Brock RW, Harris KA, et al. Cytokines contribute to early hepatic parenchymal injury and microvascular dysfunction after bilateral hindlimb ischemia. J Vasc Surg. 1999;30:533–541.

39. Wu YT, Tan HL, Huang Q, et al. zVAD-induced necroptosis in L929 cells depends on autocrine production of TNFα mediated by the PKC-MAPKs-AP-1 pathway. Cell Death Differ. 2011;18:26–37.

40. Vandenabeele P, Galluzzi L, Vandenberg Berge T, et al. Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol. 2010;11:700–714.

41. Linkermann A, Green D. Necroptosis. N Engl J Med. 2014;370:453–465.

42. Lawendy AR, Bihari A, Sanders DW, et al. The severity of microvascular dysfunction due to compartment syndrome is diminished by the systemic application of CO-releasing molecule-3. J Orthop Trauma. 2014;28:e263–e268.

43. Motterlini R, Otterbein LE. The therapeutic potential of carbon monoxide. Nat Rev Drug Discov. 2010;9:728–743.

44. Motterlini R, Haas B, Foresti R. Emerging concepts on the anti-inflammatory actions of carbon monoxide-releasing molecules (CO-RMs). Med Gas Res. 2012;2:28.