Phonomyography as a noninvasive continuous monitoring technique to diagnose acute compartment syndrome

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DEDICATION

This document is dedicated to my family, who are my pillar, my guide and an example of life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Purpose: In acute compartment syndrome (ACS), clinicians have difficulty in diagnosing soft tissue hypoperfusion provoked by the increased intra-compartmental pressure in a timely and non-invasive manner. Once identified, immediate surgical intervention is required to relieve the pressure; left untreated, it may cause loss of limb function. Phonomyography, a method that detects low-frequency acoustic signals; produced by muscle contraction, is commonly used by anesthesiologists to evaluate neuromuscular blockade in the setting of general anesthesia. To our knowledge, it has not been employed as a tool to detect acute compartment syndrome. We hypothesize that alterations in muscle contraction caused by tissue hypoperfusion in ACS can be detected with phonomyography, which would thus serve as a reliable non-invasive technique in the detection of this condition.

Methods: An established ischemic model of limb injury in Sprague-Dawley rats was used to assess the efficacy of phonomyography in diagnosing ACS. Fifteen rats were tested within a standard duration of injury: 30 minutes, and 1, 2, 4, and 6 hours. The right leg served as a control while the left leg was used to evaluate the model. Percutaneous nerve stimulators were inserted adjacent to the sciatic nerve in the buttock region (confirmed by muscle contraction evidence in the limb) and phonomyography microphones were placed over the posterior calf of both limbs. Percutaneous nerve stimulation at 10-minute intervals provoked muscle contraction in the affected and control hind limbs that was evaluated using a patented phonomyography device. Comparison between phonomyographic output, and duration of injury in each leg was performed. Muscles (tibialis anterior and extensor digitorum longus), and nerves (sciatic and posterior tibialis nerves) biopsies were taken from the injured and control limbs for routine histological analysis. Correlation was then made between duration of injury, phonomyographic output and degree of muscle and nerve necrosis in the affected limb, using the contralateral limb as a control. The data was analyzed with Wilcoxon signed rank test.

Results: The Phonomyography signal showed a significant decrease in the low frequency signal output from injured muscle, which correlated with the duration of muscle and nerve ischemia, as well as histologic necrosis. There was a statistically significant ($p<0.05$) decrease of the phonomyography signal at all-time points of ischemia, except for the 6 hours of ischemia ($p= 0.109$). After 30min of ischemia, the phonomyography signal decreased 45% ($n=15$; $p=0.005$), corresponding with rare nerve and muscle necrosis (graded by an expert veterinary pathology). Following 1 hour of ischemia, the signal decreased 55% ($n=12$; $p=0.005$), corresponding with 5-10% of muscle necrosis. Two hours of ischemia resulted in a signal decrease of 76% ($n=9$; $p=0.015$), corresponding to 100% of muscle necrosis but little nerve damage. After 4hrs of ischemia the signal decreased 86% ($n=6$; $p=0.028$), corresponding to 100% of muscle necrosis and severe nerve damage. Following 6hrs of ischemia, there was a decrease of phonomyography signal of 95% ($n=3$; $p=0.109$), corresponding to 100% muscle necrosis and severe nerve degeneration.

Conclusion: Changes in phonomyography signals were able to detect early ischemic injury in the setting of ACS prior to the onset of nerve or muscle necrosis. Phonomyography appears to be a promising non-invasive technique to detect early changes in muscle physiology following acute ischemia. Further testing is needed to evaluate its potential use in humans.

ABSTRACT

Objectif: Dans le syndrome des loges aigu (ACS), les cliniciens ont de la difficulté à diagnostiquer l'hypoperfusion des tissus mous provoqués par l'augmentation de pression en temps opportun et de façon non-invasive. Une fois identifié, une intervention chirurgicale immédiate est nécessaire pour réduire la pression; si elle n'est pas traitée, elle peut entraîner la perte de la fonction du membre. Phonomyographie, une technique qui détecte les signaux acoustiques de base fréquence produites par la contraction musculaire, est actuellement utilisé par les anesthésistes pour évaluer le blocage neuromusculaire dans le cadre de l'anesthésie générale. À ce jour, cette technique n'a jamais été utilisée comme un outil pour détecter le syndrome des loges aigu. Nous émettons l'hypothèse que des altérations de la contraction musculaire provoquée par l'hypoperfusion tissulaire dans le syndrome des loges aigu peuvent être détectées avec phonomyographie, qui pourrait donc servir comme une technique non invasive fiable dans la détection de cette condition.

Méthodes: Pour tester l'efficacité de phonomyographie, nous avons utilisé un modèle ischémique établi de blessure à un membre de rats Sprague-Dawley. Quinze rats ont été testés avec une durée d'ischémie standard: 30 minutes et 1, 2, 4 et 6 heures. La jambe droite a servi de témoin et celle de gauche pour évaluer le modèle. Des neurostimulateurs transcutanées ont été placés près du nerf sciatique dans les fessiers et les microphones de phonomyographie ont été placés sur le mollet postérieur de deux membres. Une stimulation nerveuse transcutanée à intervalles de 10 minutes a provoqué la contraction musculaire dans les mollets qui a été évaluée en utilisant un dispositif de phonomyographie breveté. La corrélation entre le signal phonomyographique et la durée de l'ischémie a été effectué. Des biopsies musculaires (jambier antérieur et extenseur digitorum) et nerveuses (sciatique et tibial postérieur) ont été récoltés au niveau des loges des 2 jambes pour une évaluation l'histologique pour évaluer l'étendue des lésions nerveuses et musculaires résultant de l'occlusion artérielle. Une corrélation a ensuite été faite entre la durée de l'ischémie, le signal phonomyographique et le degré de nécrose musculaire et nerveuse.

Résultats: Les données ont été analysées avec Wilcoxon Signed Rank test. La phonomyographie a démontré une diminution significative de signal qui était en corrélation avec la durée de l'ischémie et la nécrose histologique. Dans tous les points de temps d'ischémie, il y avait une diminution significative ($p<0,05$) du signal de phonomyographie, avec l'exception de 6 heures d'ischémie (possiblement dû à la quantité d'animaux utilise n=3). Après 30 minutes d'ischémie, le signal a diminué 45% (n=15,p=0,005), correspondant à des très rares lésions nerveuses et 1 % de nécrose musculaire. Suite a 1 heure d'ischémie, le signal a diminué 55% (n=12, p =0,005), ce qui correspond à 5-10 % de nécrose musculaire. Après 2h d'ischémie, le signal a diminué 76% (n=9, p=0,015), ce qui correspondait à 100% de nécrose musculaire mais de rares lésions nerveuses. Suite à 4h d'ischémie, le signal a diminué 86% (n=6, p=0,028), ce qui correspondait à un maximum de 100% de nécrose musculaire et de sévère lésions nerveuses. Après 6h d'ischémie, il y avait une baisse de signal phonomyographique de 95% (n=3; p: 0,109), correspondant à 100% de nécrose musculaire et sévère dégénérescence nerveuse.

Conclusion: La phonomyographie est une technique non invasive prometteuse pour détecter les premiers changements physiologique des muscles qui se produisent à la suite du syndrome des loges aigu, en démontrant une modification dans le signal acoustique émis par les muscles ischémiques. Des futurs essais sont prévus pour évaluer son utilisation potentielle chez l'humain.

CHAPTER 1 Introduction and Historical Background

1.1 History of Acute compartment Syndrome (ACS)

Acute compartment syndrome of an extremity is an orthopaedic emergency. It is a condition characterized by increased pressure within a single or multiple muscle compartments. Muscle compartments are groups of muscles surrounded by fascia, a non-compliant tissue. Within a compartment there are muscles, nerves, arteries and veins. When the pressure increases in the compartment, the vascular supply to the muscles and nerves is decreased due to collapse of small calibre components of the venous and arterial systems, causing ischemic changes in the limb. Missed acute compartment syndrome has devastating effects for patients due to fibrosis of the nerves and muscles and consequent numbness, contracture and deformity. Once identified, immediate surgical intervention is required to relieve the pressure by means of fasciotomy; left untreated, it may cause loss of limb function.

In the nineteenth century, Dr. Richard von Volkmann (1830-1889) was the first to describe the sequelae of acute compartment syndrome in his paper “Non-infective Ischemic conditions of various fascial compartments in the extremities”. Volkmann noticed this entity after applying a tight bandage to an injured limb; he observed that the paralytic contracture could be present early after the trauma causing arterial insufficiency and muscle ischemia (1, 2). He described a contraction of the flexor muscles of the hand due to ischemia in the forearm, with the presence of massive venous stasis and arterial insufficiency caused by a tight bandage. Later it was determined that muscle fibrosis was causing the contracture as a consequence of the ischemic process and inflammation(3, 4).

In 1888 Petersen(5) was one of the first to describe fasciotomy in patients with forearm contracture; he reported some improvement in the function of the upper extremity after releasing the scarred tissue.

The term Volkmann's ischemic contracture was first used in 1906 by one of Volkmann's student, Hildebrand, and was described as an entity that would develop as a consequence of untreated compartment syndrome, resulting in a characteristic deformity of the volar aspect of the forearm. He mentioned as poor prognostic factors the muscle and nerve injury present in the contracture. He also noted the association of an elevated pressure in the tissue and ischemic contractures, affecting the venous return and vascular supply (6, 7).

Thomas published a review in 1909 of what was known to that date about Volkmann's ischemic contracture. He reviewed 112 cases and noticed an association between compartment syndrome and upper limb conditions such as contusion, fractures, arterial injuries, embolus, and tight bandage causing external compression (8).

The first person to suggest that part of the injury was due to reperfusion was Rowlands in 1910(4). He mentioned that muscle and nerve edema and congestion following prolonged ischemia could explain the acute compartment syndrome. Later, in 1914, Murphy noticed that after a fracture of the forearm the tension deep to the fascia increases, causing cyanosis of the arm and hand. He reported that an increase of inflammatory products could further increase the pressure and cause cellular destruction. Murphy was the first to suggest that performing a fasciotomy (surgical release of the surrounding fascia), when symptoms persisted could prevent the development of a contracture in the forearm(9). Murphy was the first to establish the link between increased compartment pressure, muscle contracture and fasciotomy as a treatment.

In the effort of better understand the cause of acute compartment syndrome, Brooks in 1922 (10) posited that Volkmann's ischemic contracture was a result of acute venous obstruction, ultimately decreasing tissue perfusion;

Blick and Schwartz in 1986 and 1989(11, 12) described in his article a high incidence of acute compartment syndrome in high-velocity gunshot wounds causing fractures of upper and lower extremities seen during the World War II. In 1958 Ellis (13) reported a 2%

incidence of contractures after tibial fractures, drawing attention to the fact that ACS affected not only the upper but also to the lower extremities. It was thought that the vascular spasm created by the high-velocity injury was responsible for the ACS (3); the aim of the treatment at this time was to relieve the arterial spasm rather than performing a fasciotomy for decompression.

In 2000, McQueen(14, 15) reviewed 164 patients that presented to a single centre over an 8 year period with ACS and concluded that men had a higher incidence of ACS than women and that certain injuries where more prone to ACS than others. Most patients were men with a mean age of 30 years. An average annual incidence of 7.3 per 100 000 for men and 0.7 per 100 000 for women was found. There was an associated fracture in 69% of cases. The most common fractures associated with ACS were tibial shaft fractures (36%) and distal radial fractures (9.8%). In 23% there was no associated fracture but only soft tissue injury. Motor vehicle accidents were the most common cause of injury followed by sports. Additionally, they noticed 1/3 patients had either a bleeding disorder or were on anticoagulation medication. They recommended a high index of suspicion in patients with fractures of the tibial diaphysis, patients with high-energy injury to the forearm diaphysis or distal radius, and patients with high-energy fractures of the tibial metaphysis.

Due to the previous hypothesis described above, much of the research focussed on how to resolve the arterial spasm as a treatment for ACS. Patman and Thompson (16) reviewed 164 patients who underwent arterial reconstruction for peripheral vascular disease with subsequent fasciotomy. They concluded that fasciotomy should be performed more often after arterial blood flow restoration in an ischemic extremity in order to increase the chances of limb salvage. This finding was corroborated during the Vietnam War where it was found that fasciotomy after arterial repair improved long-term results (17).

ACS is not exclusive of upper extremity and leg. It has been described in the foot after Lisfranc injuries (18-23), as well as the thigh and gluteal compartments in polytrauma patients (24).

Prior to 1966, there was no clear understanding of the anatomy or the actual number of compartments in each limb. Seddon (25, 26) was the first to demonstrate the existence of four compartments in the lower extremity and the potential need to decompress all of them in the setting of ACS. Many authors have subsequently confirmed these initial findings (27, 28).

There has been a lot of research done to understand the pathophysiology of ACS. It is known that the increased compartment pressure creates an increase in the extraluminal pressure greater than the intraluminal pressure of vessels within the affected compartment. This provokes collapse of small calibre vessels (including capillaries, venules and arterioles) thereby decreasing blood flow. The lack of adequate blood flow results in local tissue hypoxia and an increase in capillary permeability causing exudation and edema. All these changes further contribute to increases in the compartment pressure, setting up a vicious circle (27).

Mubarak and Hargens in 1981 (27-29) noted that osseofascial boundaries present in the musculoskeletal system play an important role in maintaining the pressure in the compartment and contributing to compartment syndrome.

Severe functional deficits may result from missed or undiagnosed ACS due to muscle necrosis, contracture and irreversible muscular and neuronal damage (30, 31). Delayed fasciotomy exposing necrotic tissue to bacterial pathogens can result in sepsis and amputation(32). Sheridan and Matsen in 1975 (33) underlined that the incidence of complications decreased with early diagnosis and subsequent fasciotomy.

1.2 Military wounds and acute compartment syndrome

Missed acute compartment syndrome can have devastating functional sequelae. This condition is of particular interest in military combatants because the high-energy mechanism of their injuries places them at greater risk of ACS.

Prophylactic fasciotomy is routinely performed in military patients to prevent the complications and limb impairment associated with a delay in diagnosing ACS. Furthermore, prophylactic fasciotomy is often performed to ensure adequate limb perfusion during the transport to a level four facility where military patients can receive definitive treatment of their injuries. It takes at least 6 hours to transport a patient from the combat area (Iran/Afghanistan) to Landstuhl Regional Medical Center in Germany (LRMC is the largest tertiary care US military medical facility outside the United States.) (34).

"In the current chain of evacuation of injured warriors from the theater of combat operations, patients are flown from Iraq or Afghanistan to LRMC in Germany, the military's only level 4 facility and the largest tertiary care US military medical facility outside the United States. Timing of transport to LRMC is variable but often commences within 24 hours of injury, depending on transport availability and patient condition. This flight time from Iraq to Germany is approximately 6 hours, and additional time is necessary to load and unload patients from the aircraft on either end. During this time, access to an operating room for repeat evaluation or repeat surgical debridement of a limb is not possible. Surgeons must recognize these challenges, and they should have a low threshold for release of compartments prior to patient transport whenever any suspicion of potential for compartment syndrome is present. Prolonged recumbence, relative immobilization, and ongoing fluid resuscitation all may contribute to impaired limb perfusion. No primary skin closures should be performed in theater, and bulky dressings should be used to assist in collecting the substantial drainage that may occur from wounds during transport."(34, 35)

There is a 15% incidence (36) of compartment syndrome in military patients due to high-energy injuries in combat, mostly by explosives. In a retrospective study by Ritenour et al., patients who underwent fasciotomy in Iran, Afghanistan, or Landstuhl Regional Medical Center (LRMC) with delayed diagnosis of ACS had significantly higher rates of muscle excision, amputation and mortality. It has been reported that in military conflicts, more than 71% of blast injuries cause severe extremity trauma, with 86% of the victims requiring fasciotomy (36-39).

In 2010, Kirk et al. (35) provided a very clear reasoning for prophylactic fasciotomy done before aeromedical evacuation of military patients at the battlefield. Routine prophylactic fasciotomy is done when there is clinical suspicion or when the mechanism of injury suggests that ACS may develop in the next 24 hours. Surgical theaters in combat areas have a high number of patients and its main objective is to stabilize the patient for evacuation to a higher level of care. Germany is the only level-four facility for these patients and often the transport times from the battlefield to Germany, and then to the USA, may take at least 12 to 24h. The patients are transported with analgesia and sedation, masking the diagnosis of ACS.

Missed ACS results in muscle and nerve dysfunction in the affected limb, leading to significant morbidity and, rarely, mortality(39). Landstuhl Regional Medical Center reported 13% of all injured combatants in Operations Enduring and Iraqi Freedom (January 2005 and August 2006) underwent fasciotomy for diagnosed, threatened or potential ACS (36). The high incidence of extremity injuries, with potential risk of ACS, in the Iraq and Afghan conflicts has given great importance to early diagnosis and treatment of ACS (35, 40).

Unfortunately, there is presently no effective continuous non-invasive method for detection of ACS. As a result of this inability to reliably monitor injured combatants for the development of ACS, the American Department of Defense has adopted liberal indications for prophylactic fasciotomy in injured soldiers considered at risk (41) especially those requiring prolonged transportation for treatment. This approach is supported by

recent data from the Iraqi conflict that has shown that delayed and/or inadequate fasciotomy results in a three- to four fold increase in mortality and twice the rate of major amputation (36). However, the drive to reduce the incidence of ACS with prophylactic fasciotomy must be weighed against complications associated with it: increased risk of infection, increased operative time and blood loss in patients who are often physiologically unstable, and limitation of surgical-approach options for any later definitive procedures.

Given the high incidence of ACS in present day combat, the significant risks of missing or delaying the diagnosis, the risk/limitations inherent in prophylactic fasciotomy, and the absence of a reliable non-invasive continuous method of monitoring for it, it is imperative a that such a device be developed.

Phonomyography represents a potential solution to this problem. By employing surface electrodes for nerve stimulation combined with surface microphones overlying the compartments at risk, it can potentially provide non-invasive and continuous evaluation of muscular contractile function. The electrodes and microphones can be applied beneath dressing and/or immobilization material if necessary. Once muscular physiological dysfunction is detected, early immediate management of the compartment syndrome can be instituted.

Benefits of early detection and treatment of ACS include: 1) reduction in severity of secondary injury to the combatants; 2) facilitation of surgical interventions; 3) reduction in requirements for rehabilitation and long term care; 4) more rapid reintegration of military personnel into the workforce and; 5) reduction in costs to the military.

Chapter 2 Acute Compartment Syndrome: Pathophysiology, Diagnostic Methods

2.1 Acute compartment syndrome, Pathophysiology

Acute compartment syndrome involves the increase of pressure within the muscle compartments of limbs that impedes adequate vascular supply resulting in muscle and nerve ischemia and subsequent necrosis, if not identified and treated with fasciotomy prior to the onset of irreversible ischemia. These changes alter the relation between the volume in the compartment and the pressure inside. (42). Figure 1.

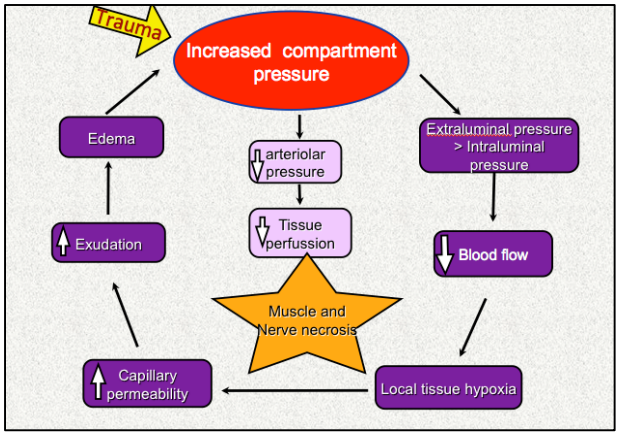


FIGURE 1: Pathophysiology of ACS, Modified from: Campbell's Operative Orthopaedics (43).

Acute compartment syndrome can result from any condition that increases the content or reduces the volume of a compartment. It can follow a fracture, blunt trauma that results in excessive edema, haemorrhage, or vascular obstruction. As well, burns increasing capillary permeability associated with massive volumes of fluid resuscitation, volume constriction such as a tight cast or dressing, and the revascularization- reperfusion injury syndrome are all possible causes of ACS (44). Other etiologies include crush injuries, prolonged external compression, third degree burns, snake bites, and intravenous regional anesthesia (45).

Rorabeck et al(46) described that no matter the cause of ACS, the intra-compartment pressure does not increase sufficiently to totally obstruct the systolic and diastolic pressure of main vessels. This means that in the clinical diagnosis of ACS, a pulse is typically palpable distal to the affected compartment as the compartment pressure rarely

exceeds the systolic blood pressure of the large bore arteries coursing within the affected compartment (47, 48).

McQueen(15), in her study of 164 patients with ACS followed for 8 years noticed that the most important clinical parameter is the difference between diastolic blood pressure and intracompartmental pressure (*delta pressure (*Δp)). A difference of less than 30mmHg was felt by the authors to be the critical threshold for the onset of ACS Whiteside theory: DBP (Diastolic blood pressure) - CP (compartment pressure) should be greater than 30 (DBP-CP= more than 30 ideal)(49).

Multiple experiments in animal models have been performed to better understand the ischemic process and tissue injury seen in ACS. It has been shown that, during ACS, muscle blood flow is decreased during ACS (50, 51). Lawendy et al demonstrated with intravital videomicroscopy (IVVM) that there is a “microvascular dysfunction” in ACS, with a decrease in capillary perfusion and an increase in cellular injury after 45min of ischemia (44).

Nouri et al 2008, showed in an animal model that after 3 hours of limb ischemia, there was specific nerve damage observed by light microscopy; at 4 hours of ischemia axonal swelling was present and nerve damage was progressive. Maximal ischemic changes were observed at 7 days with fiber degeneration and axonal swelling (52).

Compartment pressures are currently measured using an invasive and non-reliable technique when ACS is clinically suspected (53). The pressure in the compartments is measured by inserting a needle through the skin and the fascia. Different companies have developed specialized equipment to measure the compartments such as the *Striker* and *Synthes* compartment pressure equipment.

Unfortunately, there is a risk of introducing bacteria and causing an infection every time the skin barrier is broken either with a wound or a needle to measure compartments. Also, the needle measures only one compartment and requires re-insertion into the different

compartments when there is the clinical suspicious further increasing the probability of infection. For example, the lower leg has 4 compartments, the thigh has 3 compartments and the forearm has 4 compartments. At the same time once the needle is inserted the measurement can change depending on position and distance from the zone of injury, resulting in a false negative measurement.

Compartment pressures can also be measured with an arterial line. A specific monitor and arterial line equipment are required; these are not always available in rural areas or non-trauma centers. A needle is inserted in the compartment to measure the pressure and the delta pressure is calculated using the diastolic pressure of the patient.

When ACS is clinically suspected, the management is surgical consisting of fasciotomy of the affected compartments regardless of whether the pressures have been measured. The definitive treatment of ACS is to decompress the pressure within the compartments by incising the skin and underlying fascia, a procedure known as fasciotomy. In emergency situations where there are no proper operating facilities, fasciotomies can also be done by the bedside. In war zones where there are no operating rooms this is done in primary health areas with minimal resources.

In 2007, Semenov reported that a thorough clinical examination is the most sensitive method to early diagnose ACS in most patients. However, the clinical examination is unreliable in obtunded, head injured or critically ill patients (54). Objective data is required in these situations and any measurement must be accurate and reproducible to ensure an accurate diagnosis. Currently, pressure measurements are our best means of determining the need for fasciotomy although clinicians are unable to reach a consensus as to the critical pressure threshold. In addition, these tests are invasive, technique dependent and position sensitive.

“The damaged muscle progressively degenerates creating a non-regenerative environment remains”(55). The reperfusion causes more injury than the actual ischemia. In the ischemic period, free oxygen radicals are produced causing an increase in swelling.

Releasing the pressure through the surgical intervention of fasciotomy prevents further damage but does not reverse the damage already done; it allows debriding the necrotic tissue and inspecting the compartment's condition.

2.2 Acute compartment syndrome, Diagnostic Methods

Although the diagnosis of ACS is mostly clinical using physical examination, there are additional diagnostic tests that can be used to correlate with the signs and symptoms by directly measuring compartment pressures. However, the clinical diagnosis is not always easy and the compartments pressure measurement is not always reliable.

The classic clinical findings are pain on passive stretch of the muscles contained within the affected compartments, pallor, pulselessnes, and paresthesia. Tense compartment and paralysis have also been described. Unfortunately, the presence of all these signs only occurs in late ACS after there has already been irreversible muscle and nerve injury. The presence of an open fracture does not exclude the possibility of ACS. Blick 1986 and Christensen 1985 have shown that up to 9% of open tibial fractures can be complicated with ACS (11, 56). Mubarak et al (57, 58) noticed that some patients may develop muscle necrosis after a missed ACS or undiagnosed ACS. A summary comparing the diagnostic methods that are used today is found in table 1.

Lynch et al 2004, mentions that up to 50% of patients with clinical presentation of ACS did not correlated with measured elevated compartment pressures, mentioning in his study that if only the clinical presentation is used to diagnose ACS this may lead to over diagnosis and unnecessary surgery (59). Shuler et al, found that the probability of ACS with one clinical finding was approximately 25%, increasing to 93% with more than 3 clinical findings present (60).

PAIN

Pain is the main clinical presentation when patients present with ACS. The pain is often out of proportion to the injury and does not subside with pain medication. However, making the diagnosis of ACS is challenging in the unconscious or obtunded patient given that pain can be masked and is difficult to assess. Pain and tense swollen compartment is often the earliest sign, however this can be present in a limb injury without compartment such as a fracture or crush injury. Currently, the most reliable clinical sign is pain on passive stretch of the affected muscles and it is also the earliest sign to be present.

TENSE COMPARTMENT (palpation)

Shuler et al 2010 compared the current diagnostic methods for ACS between different staff and residents, reporting a poor correlation of manual detection of compartment firmness associated with elevated intracompartmental pressure. A sensitivity of manual palpation of 24%, with 55% specificity, negative predictive value of 63% and positive predictive value of 19% were found(60). Ulmer et al, reviewed over 500 articles and found a low sensitivity (13% -19%), a positive predictive value of the clinical findings of 11% to 15%, a specificity of 97% and negative predictive value of 98%.

PULSELESSNES

Rorabeck studied 18 patients with lower extremity injury including either tibia fractures alone or associated with fibula fractures where present. Pain on passive stretch was the most prevalent sign. Interestingly pulses were present in all the patients except one patient who had a vascular injury(46). He also noted that most of the sensory deficit represented increase pressure in more than one compartment and was “not a helpful sign in differentiating the compartment involved.” If there is a question of arterial injury, it should be assessed with an ankle brachial index. The clinical presence of pulse and good capillary fill does not exclude ACS.

The diagnosis is more challenging in the polytrauma patient. These patients often present with drug or alcohol intoxication, associated head injury, or require endotracheal intubation, sedation and the use of specific paralyzing or anesthetic agents. The

threshold for ACS can be affected in patients with low blood pressure where they can develop ACS with lower pressures within the compartment than the pressure required if the same patient where to have normal blood pressure. In these cases, it may help to measure the compartment pressure of the suspected compartment or all the compartments This can be a complex task as there could be difficulties in continuous monitoring of the pressures requiring leaving the needles in place or puncturing multiple times each compartments.

There have been several studies that have shown that different anaesthetic methods may mask an impending ACS delaying its diagnosis and putting the patient at risk of limb functional deficit. Harrington et al noted “The administration of intravenous morphine via a PCA pump also has the capacity to eliminate the most significant symptom of incipient compartment syndrome, namely, pain” (61). Uzel et al in 2009, showed that “When nerve blocks are used, they should be more analgesic than anesthetic. Careful patient monitoring remains important” (62)

Mar et al, in a systematic review reported that patients who underwent epidural analgesia, 32/35 had classic signs of ACS. They concluded that pain control and anesthesia should not affect the clinical presentation or diagnosis of ACS. They mentioned that there is no convincing evidence that patient-controlled analgesia opioids or regional analgesia delay the diagnosis of ACS, and they emphasize that patients should be monitored. They strongly recommend that regardless of the type of analgesia used, a high index of clinical suspicion, ongoing assessment of patients, and compartment pressure measurement are essential for early diagnosis (45). The view that analgesia should be withdrawn or an inferior mode of analgesia be used to facilitate diagnosis of compartment syndrome should be discouraged.

More recently in 2013 Stephen showed that “A review of the available evidence suggests that regional anesthesia should be offered to patients at risk for ACS. Continuous techniques, particularly continuous peripheral nerve blocks appear to be safe” (63).

TISSUE PRESSURE MEASURING TECHNIQUES

Needle manometer:

Landerer in 1884 (53, 64) was the first one to measure the compartment pressure. The first ones to apply the needle manometer to diagnose ACS were Whitesides et al. The technique is described with an "18-gauge needle connected to a 20 ml syringe by a column of saline and air", and this "column should then be connected to a mercury manometer"(49). They describe that after the needle enters the compartment, "the air pressure in the syringe raised until the saline-air meniscus was seen to move and the mercury manometer would measure the pressure" (49), (Figure 2). Rorabeck (31) showed that this technique is not always reproducible and cannot be used for continuous monitoring of compartment pressures (50).

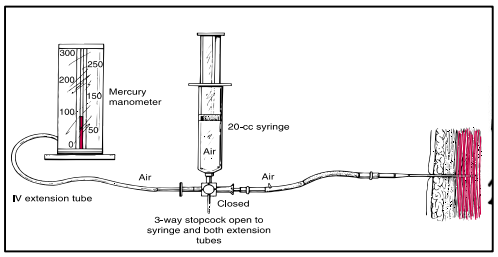


FIGURE 2

FIGURE 2: Mercury Manometer.
Adapted from Whitesides et al (65).

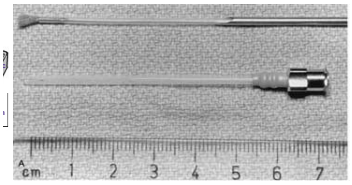
Matsen et al (42) modified the needle manometer technique to allow for continuous monitoring of compartment pressures. This required continuous infusion of normal saline into the compartment (0.7cc/day rate of saline infusion). A transducer recorded the pressure required to infuse saline, measuring tissue resistance. Later Mubarak et al (80) found that tissue compliance (which is reduced at pressures more than 30mmHg), may have an effect on the accuracy of the technique. Rorabeck et al(31) noted that this infusion technique tends to give artificially high readings especially if the infusion pump

erroneously delivers more saline than indicated and causing an ACS (by infusing in the compartment and increasing the pressure intracompartmental).

Wick Catheter

Scholander et al (66)developed the first technique to measure intracompartmental pressures that did not rely on continuous infusion. The tip of the catheter has a piece of polyglycolic acid suture through PE60 polyethylene tubing. This technique was then applied in clinical cases (57, 67-69).

The wick catheter, as it was described, uses a pressure transducer and recorder while the accuracy of the technique depends on not having any air bubbles in the tubing system as this may create artificial low readings (Figure 3). A large trocar is used to insert the catheter in the compartment and the catheter is taped to the skin. Although it is a good technique for continuous monitoring, it has some disadvantages: the tip of the catheter may block by a blood clot, the polyglycolic acid may hydrolyze, only a fine suture is holding the wick into the tubing, the suture could displace and the wick would remain in the tissue.



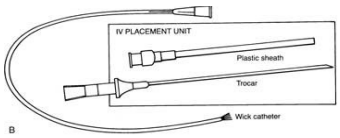


FIGURE 3:
Wick catheter (53).

FIGURE 3

Slit catheter

Rorabeck et al, first developed the Slit catheter technique (31, 46). This technique consists of a PE60 polyethylene tubing and five 3mm slits at the end (decreasing the risk of leaving the catheter in the tissues). It requires a 16-gauge needle, a catheter and a transducer. The catheter is filled with normal saline and similar to the Wick catheter, this

technique requires absolutely no air bubbles within the system. It requires calibration of the monitor by zeroing the system when placing the tip and the transducer at the same level. The catheter is also taped to the skin. There is an alarm that can be set when a certain pressure is reached. Pressing on the compartments (plantiflexion or dorsiflexion), changes the monitor readings, which can also make the reading unreliable. (Figure 4,5)

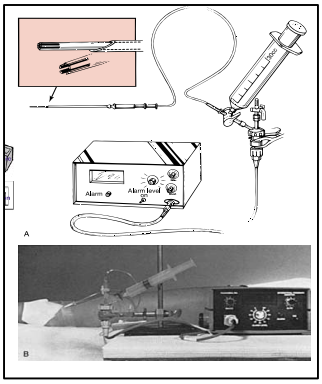


Figure 4

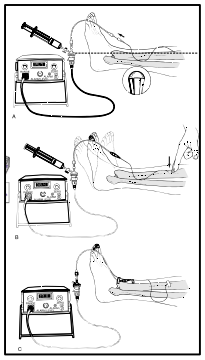


Figure 5

FIGURE 4: SLIT CATHETER system. Adapted from Mubarak et al (57).

FIGURE 5: Slit catheter and changes in measurement with manipulation from Twaddle et al(53)

Stic Catheter (Stryker)

Manufactured by Stryker, the Stic catheter is frequently used to measure pressures in the acute settings. It requires a disposable syringe prefilled with saline and a disposable needle-catheter. The system needs to be purged to avoid air bubbles in the system and then it is zeroed at the level of the compartment. The needle is inserted through fascia and the monitor gives a measure of compartment pressure. In the clinical setting, the readings of the system can continue to drop with time and individual variations may affect the measurement.

Boody and Wongworawat 2005 (70), found that the Stryker monitor system was accurate in measuring in vitro models of known pressure. When comparing different types of needles, they found that the slit catheter was the most accurate while the side port needle was found to be less accurate. They noted that the straight needle tends to overestimate the pressure. They believed the slit catheter minimizes tip occlusion and increases the surface area at the catheter-tissue interface(71). (Figure 6.)

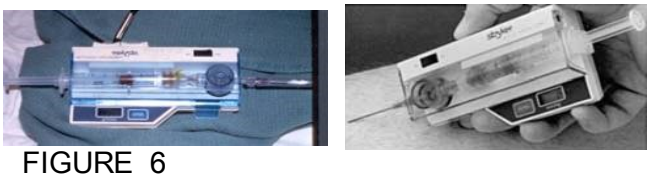


FIGURE 6

FIGURE 6: STIC catheter Stryker Mississagua Ontario, Canada.

The needle positioning and angle in which it is inserted affects the reading of the compartmental pressure. The pressure should be measured with a relaxed extremity and the knee and ankle should be in a relaxed position during the serial recordings. There is no standard position for needle insertion but some authors suggest a 45 degree angle to the skin(72) .

MICROCAPILLARY INFUSION TECHNIQUE

Styf and Korner(73) described this technique essentially to diagnose chronic compartment syndrome, useful for long term monitoring, with dynamic applications.

Arterial transducer measurement:

A catheter measures the arterial pressure using a 16-gauge catheter; it is flushed with saline and connected to the pressure monitor. The catheter is inserted in the compartment

of interest and the arterial line monitor is calibrated at the same level of compartment to give a read of the intracompartmental pressure.

Barker et al (74) described with precision the technique for measuring compartment pressures with a pressure catheter and an arterial line pressure monitoring with a transducer. This is a variation of other techniques as they mention that the Slit catheter is expensive and not always available. More importantly he mentions the importance of continuous compartment monitoring as the patients' condition and blood pressure may change making even more challenging the diagnosis.

All of the above techniques have difficulties if not trained adequately to use such catheters. They are all invasive techniques that require breaking the skin barrier and do not provide reliable continuous monitoring.

NON INVASIVE TECHNIQUES

Scintigraphy

Scintigraphy using Tc 99m-methoxyisobutylisonitrile (Tc 99m-MIBI) described by Edwards et al (75) detects alterations in muscle perfusion ,which are graded with treadmill exercise. This method has been used as a screening tool for invasive pressure monitors, giving good positive and negative predictive value**.**(75).

Laser Doppler Flow

Mostly used in chronic exertional compartment syndrome (exercise related) described by Abraham et al (2).

Near-Infrared Spectroscopy (NIRS)

NIRS measures changes in tissue oxygenation (potential use: to establish normal values for ACS). NIRS is a non-invasive, continuous, and direct method of monitoring tissue oxygenation and hemodynamics. NIRS exploits the difference in optical absorption spectra between oxygenated (O2Hb) & deoxygenated (HHb) states of hemoglobin (Hb) in a tissue. Local ischemia causes an increased extraction of oxygen by muscular tissue reducing the level of O2Hb and increasing the level of Hb.

Kim et al, used NIRS in an ischemic animal model and found that NIRS signal was affected by the tourniquet applied to create ischemia. This occurred due to different amounts of HbO2 present in the ischemic and reperfusion periods, showing lower Hb concentration in the reperfusion period compared to baseline (76). Shuler 2010 et al, showed that NIRS could be useful for detecting changes in compartmental pressures in an injured leg with or without ACS. They measured for short periods of 6 seconds and on a fixed measurement area, mentioning that this may limit the study for continuous monitoring and that different pressures throughout the compartment might be missed (60, 77).

Jackson et al (2013) in their study comparing the use of NIRS to detect changes in ACS, found that skin pigmentation may affect the absolute value of NIRS. Up to 9.9% lower measurements were recorded in individuals with higher skin pigmentation, showing a negative correlation between NIRS values and melanin content (40). Near Infrared Spectroscopy (NIRS) has also shown limitations to detect the deep compartments (40, 41, 78).

MWT: 4D Microwave Tomography Functional Imaging

Semenov et al, in their 2001 study reported that the 3D in spatial domain plus 1D in the time, gives absolute anatomical images with dynamic images. This study was performed using limbs of a porcine ACS model. However, this technology is only in the early stages

and the potential costs of this equipment is high, it requires a big equipment and it is not portable (54).

Ultrasonic Device

2004 Lynch et al, used ultrasonic device for the diagnosis of ACS in cadaveric human extremities. They mention the potential use to measure sub-micrometer displacements of the fascia. However, there are adjustments to be made as Lynch et al mention, to calibrate the changes of the intramuscular pressure to possible tears in fascia that could be present and to changes due to fat layers present in obese patients and the limitation to monitor deep compartments (59).

TABLE 1. Comparison of Diagnostic methods to detect ACS found on the literature.

Comparison of Techniques	Characteristics	Authors
Manual palpation of compartments:	Sensitivity 24% Specificity 55% PPV: 19% NPV: 63%	Shuler et al (79)
	Sensitivity: 13% -19% PPV: 11%-15% Specificity: 97% NPV: 98%.	Ulmer et al (80).
Slit catheter, vs Side-ported needle vs Simple needle	18-gauge needle: produced significant higher values. Side port needle and slit catheter more accurate than straight needle.	Moed and Thorderson (81) Boody et al (70)
Accuracy of measuring techniques	16-gauge catheter (with of without side ports): produced measurements within 4-5mmHg of the slit catheter or STIC catheter readings. Slit catheter was the most accurate. Side port needle was found to be less accurate. Straight needle tends to overestimate the pressure Side port needle and Slit catheter more accurate than straight needle	Wilson et al (82) Broody(70) Pedowitz (71)

	Slit catheter minimizes tip occlusion and increases the surface area at the catheter-tissue interface.	
The needle manometer technique	With or without infusion accepts relative higher values; these values change depending on tissue compliance. In continuous infusion technique fasciotomy should be done when compartment pressures rises above 45mmHg	Mubarak (57, 69) Rorabeck (31) Matsen (30, 42, 83)
Wick, slit or Stic catheter techniques	Continuous infusion is not used, giving values threshold for fasciotomy that are lower	Mubarak (57, 69)
Arterial line manometer	Most accurate device, best correlation (R=0.9978) between calculated and measured pressures.	Boody et al (70)
Stryker device	Very accurate	Boody et al (70)
Whitesides manometer	Lacks precision needed for clinical use. Worst correlation (R=0.9115). Measures showed highest standards errors.	Boody et al (70)
Fasciotomy indications	Fasciotomy recommended when compartment pressures increases within 10-30mmHg of the patient's diastolic pressure, in a patient with clinical signs of ACS.	Whitesides et al (65)
	Fasciotomy should be done when compartment pressures rises above 45mmHg.	Matsen et al (30, 42, 83)
	Fasciotomy should be done when Slit catheter measures greater than 30mmHg.	Matsen et al (30)
	Fasciotomy should be performed when compartment pressures exceed 30-35mmHg	Mohler(84), Mubarak(69) Rorabeck (31)
	A more reliable indicator of impending ACS is the difference between diastolic pressure and the measured compartment pressure, known as Delta pressure (Δp) A difference of less than 30mmHg is surgical significant: predictive of ACS. (This study advocated this threshold only in normotensive patients with tibial fractures)	McQueen (14)

2.3 Fasciotomy

Once ACS is suspected or diagnosed, the treatment is to relieve the pressure of the compartments by means of a longitudinal incision allowing the pressure to decrease within the compartment. There is controversy as to when to do fasciotomy if the clinical presentation is not clear. Also, there are variations of acceptable normal pressure depending on the diagnostic method used. The needle manometer technique with or without infusion accepts relative higher values; these values change depending on tissue compliance. See table 1 for a summary of the different thresholds to perform fasciotomy that have been described.

Whitesides et al (65) mention that fasciotomy is recommended when compartment pressures increase within 10-30mmHg of the patient's diastolic pressure, in a patient with clinical signs of ACS. Matsen et al (30, 42, 83) recommend performing fasciotomy when compartment pressures rise above 45mmHg when using the continuous infusion technique. Amendola et al, report that measurements greater than 30mmHg with the slit catheter is a clear indication for fasciotomy (53). It has been also documented that fasciotomy should be performed when compartment pressures exceed 30-35mmHg (31, 68, 84). Whitesides et al. identified the pressure perfusion gradient at which ischemia is imminent and prophylactic fasciotomy should be done as <20 mm Hg below diastolic blood pressure (49, 65). Hagens et al, suggests an absolute threshold of 30mmHg pressure for the diagnosis of ACS(27). It has been shown by McQueen and Court-Brown that a more reliable indicator of impending ACS is the difference between diastolic pressure and the measured compartment pressure, known as Delta pressure (Δp). They recommend that a difference of less than 30mmHg warrants fasciotomy. They recommend the use of a 30mmHg or less threshold as the most reliable method of deciding when fasciotomy should be performed (14).

Heckman et al(85) mention that the distance between the fracture site and where the pressure is measured is important, calling it the "Zone of peak pressure". This distance

should be a few centimeters from the fracture site; otherwise there could be serious errors in measurement. He found that 89% had highest pressure at fracture site, 5% at 5cm distal, 2% at 5cm proximal with a difference of up to 10 mmHg. Therefore they suggest measuring the compartment pressure within 5cm of the fracture site or injury.

It is important to consider that ACS in patients who are in shock could present at lower intracompartmental pressures (49, 85). Also, when the blood pressure of the patient is too high, ACS could be present at higher pressures than usual.

Hislop & Tierney mentioned that after studying all the present invasive intracompartmental pressure and performing a physician's survey, there is no current standardize protocol. They mention that the current "Gold Standard" method for measuring the pressure in the compartment is intracompartmental, because of technical limitations and user-dependent variables the outcome of the test is not accurate. They also state that "a standard reproducible protocol for pressure testing has not been described", "this results in confusion regarding interpretation of results and reduces the tests' reliability" (72).

2.4 Humans anatomy vs Animal model

It is not uncommon to ask when doing experimental surgery projects, what animal model should I use to test and prove my project?

Different animals have been used in experimental surgery, however many things should be considered. The anatomy of the animal model should be similar to the human body in the parameters to be tested. In this study, the purpose was to use an animal model that would have similarities in the anatomy of the limbs as humans. We needed a model in which the limbs have muscles and compartments and similar physiology than humans.

It came to our attention that monkeys, pigs, dogs and rats have been used for different purposes such as limb ischemia, regeneration, and molecular analysis after limb injury

among others (44, 54). Based on this reasoning, we used Sprague Dawley rats for this study. The compartments of the limbs of the rats are very similar to humans, having similar muscular and nervous anatomy. In addition, Criswell et al(55) in their ACS animal model concluded that a rat model best mimics the sequelae of human conditions of ACS.

Chapter 3 Phonomyography

In 1665 Francesco Grimaldi discovered that when muscles contract they make a sound "acoustic myography", later known as phonomyography. It is a low frequency sound that is converted to a digital signal.

More recently the phonomyography has been used to monitor the neuromuscular blockade in anesthesia in the operating room. There is currently no reliable monitor to determine the complete picture of body muscle relaxation during surgery.

Six years ago, Dr Hemmerling started to develop a novel monitoring method, called phonomyography, which is easy to use, non-invasive and reliable to monitor all muscles of the human body. This now a patented *(US10/730,811 & CA2,415,173)* method that uses special microphones to detect the low-frequency sounds emitted by the contraction of muscles(86). The device detects these sounds and indicates via special algorithms the degree of muscle relaxation of various individual muscles.

Phonomyography has been validated in numerous studies versus mechanomyography, the latter is considered the gold standard although its use is not easy for several muscles (87, 88). The technology is already considered as a standard objective monitor for muscle relaxation during general anesthesia (89). Phonomyography is one of the monitoring parameters of *McSleepy*, the first anesthesia robot, developed by Dr. Hemmerling and his team. The application of phonomyography to detect and grade compartment syndrome is based on preliminary clinical tests, where the evoked acoustic wave was

proportional to the intra-compartment pressure: the higher the pressure the higher the amplitude. Therefore, we hypothesized that phonomyography could be an easy to use, reliable tool to continuously measure the degree of compartment syndrome.

Phonomyography is now available as a research tool, designed and manufactured by Hemmed Technologies Inc, known as the *Relaxofon.TM.*

Chapter 4 Hypothesis and Objectives

4.1 Hypothesis

Phonomyography is a reliable, non-invasive technique to detect early changes in muscle physiology that could lead to acute ischemic changes.

4.2 Objectives

Objective 1: To use phonomyography to detect early muscle dysfunction arising from ischemia as a predictor of acute compartment syndrome.

Objective 2: To correlate the sound emitted from the muscle upon contraction with the duration of ischemia as well as the degree of tissue necrosis.

Chapter 5 Materials and Methods Ischemic model

5.1 Materials and Methods

All protocols and experiments were conducted in agreement with the McGill Animal Care Committee, following the highest standards of the Canadian Council on Animal Care. The Animal Use Protocol was approved by the Facility Animal Care Committee at McGill.

Eight-week old Male Sprague Dawley rats (Charles River Laboratories) weighing approximately 400gr, were permitted 2 weeks of acclimation prior to the experiments, with food and water ad libitum. The rats were housed in an animal room under controlled conditions (temperature 22–24 °C, humidity 55–60%, 12-h light/12-h dark cycle).

Fifteen Sprague Dawley rats where randomly divided into 5 different groups based on duration of ischemia: 30min, 1h, 2h, 4h and 6h (3 rats per group). (Figure 7)

General anesthesia was induced with Isoflurane 5% and endotracheal intubation was performed to ensure adequate oxygenation and ventilation. Oxyhemoglobin monitoring with pulse-oximetry was used throughout anesthesia and recovery to monitor heart rate, oxygen saturation and respiratory rate, (MouseOx, STARR Life Sciences Corp, SLS-MO-00050). The rat was positioned in a supine position on a custom made thermoplastic-molded bed, after the lower extremities where shaved for visualization and electrode positioning. A heating lamp and heating pads maintained normothermia. Normal saline and analgesia where provided intraoperatively for hydration and pain control.

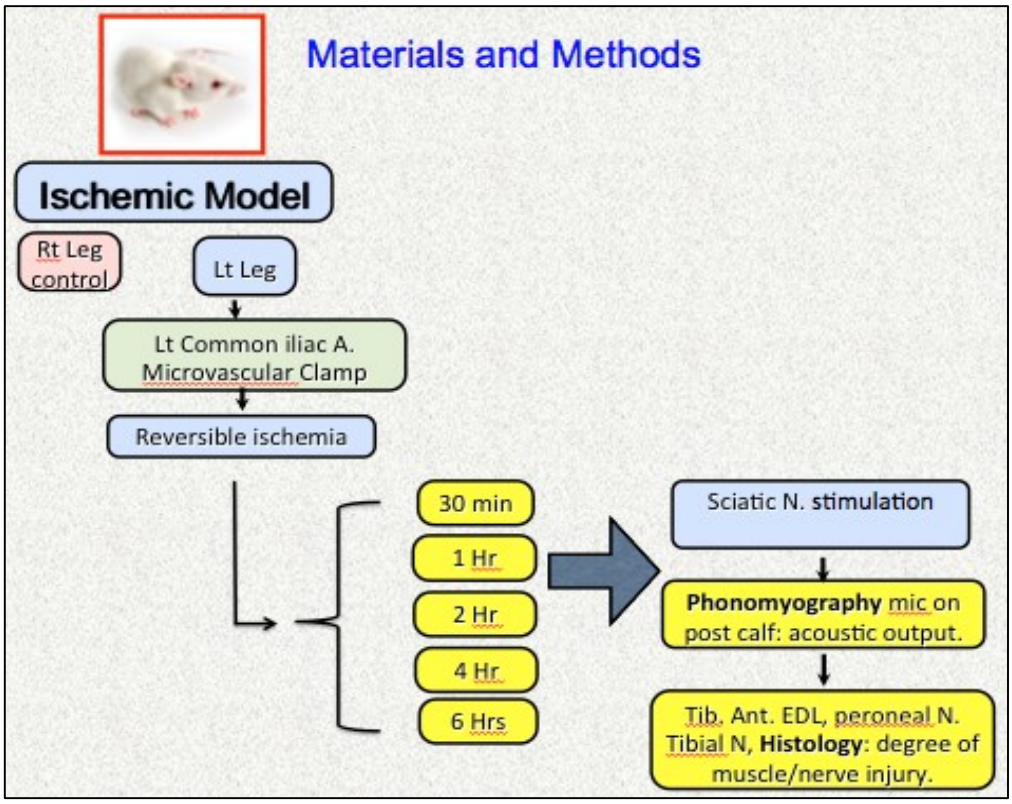


FIGURE 7: Summary of material and methods.

Ischemia was induced in the left lower extremity with the right leg serving as a control throughout. A laparotomy was performed; the left common iliac artery was identified, dissected, and reversibly clamped with a microvascular clamp (125gr=1.24N 2008 Nouri et al (52). The sciatic nerve of both lower extremities was stimulated with a nerve stimulator (*Stimuplex HNS 12, B BRAUN 6043570, B. Braun Medical Inc*) inserted in the posterior thigh. The lower extremities were held in full extension to optimize the phonomyographic acoustic signal intensity and quality. The phonomyography microphone was positioned and fixed with tape on the posterior aspect of the calves. Ischemia was confirmed by clamping the left common iliac artery under direct observation as well with immediate loss of the pulse-oximetry signal upon clamping.

The phonomyographic signal from the calf musculature provoked by Train-of –Four (TOF) stimulation of the sciatic nerve in the thigh was recorded using a commercially acquired

Relaxofon phonomyographic device (*Henmed Technologies Ltd*) every 10min during the ischemic period. Data was stored on a laptop computer.

Once the planned duration of ischemia had been attained, the microvascular clamp was removed and the leg spontaneously reperfused. The abdomen was closed with suture and the rat allowed to recover. Appropriate analgesia was given (Carprofen, according to Standard Operating Procedure) with free water and food intake for 4 days postoperative. See figures 8-11 showing the setup and experiments settings.

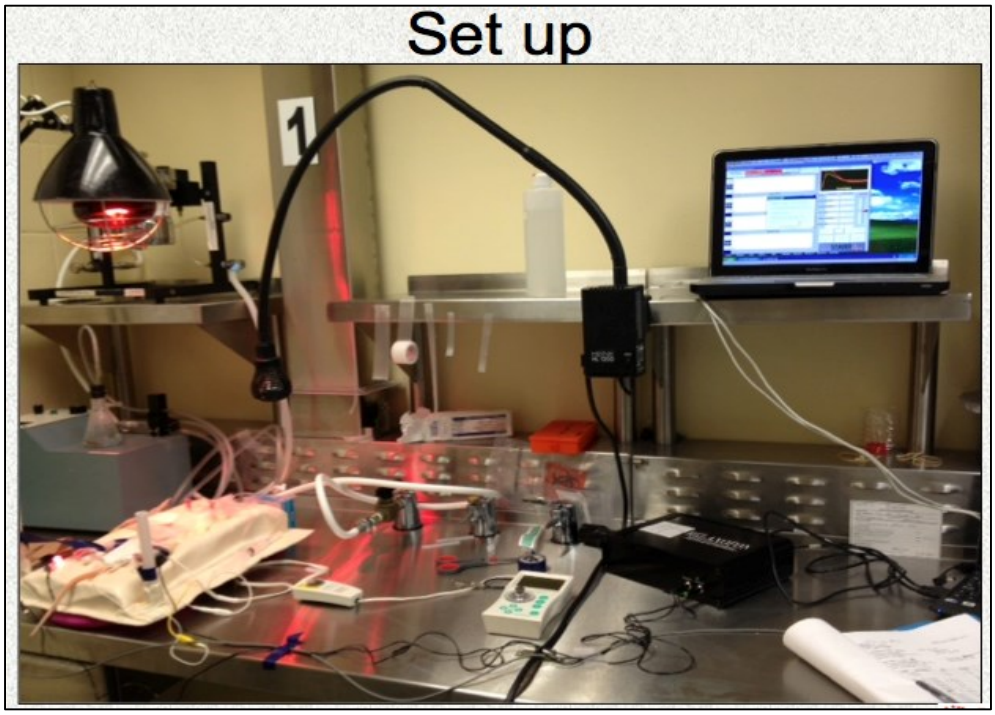


Figure 8

FIGURE 8. Setup for procedure with monitoring systems

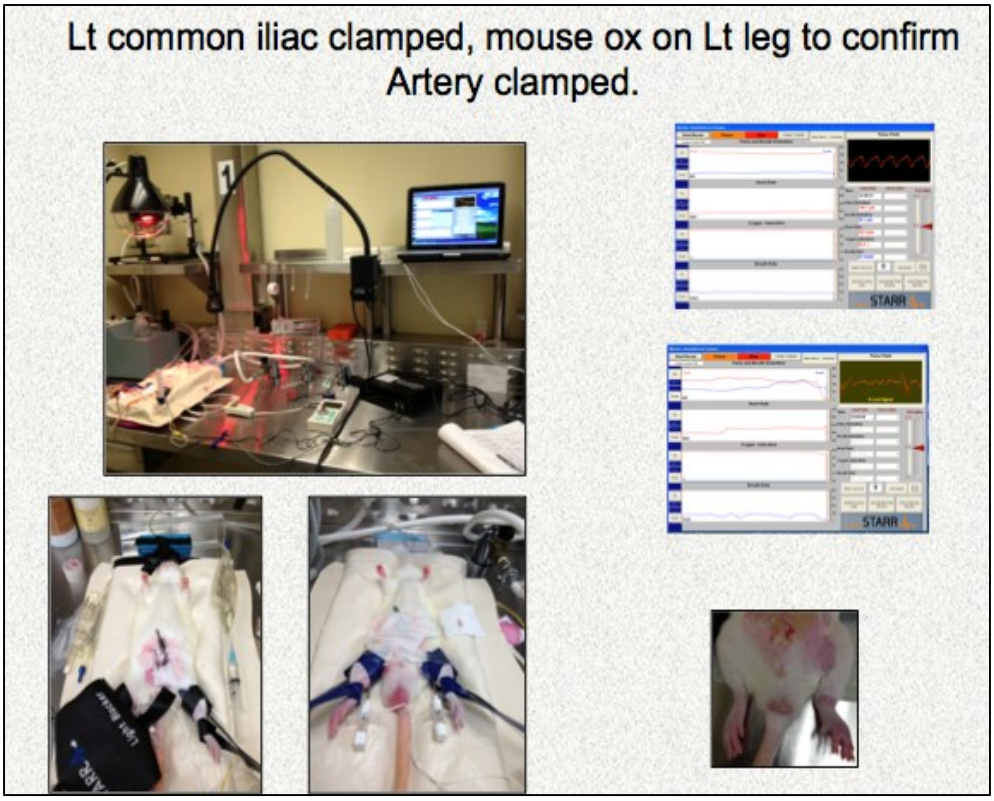


Figure 9

FIGURE 9.Left common iliac artery clamped. Top left: settings for experiments. Top right: sample of mouseOx, pulseoxymetry measurement during experiments. Bottom left: positioning of animal, laparotomy and clamping of left common iliac artery, with positioning of phonomyography, nerve stimulation and mouseOx. Bottom right: ischemic left leg of the animal post ischemia

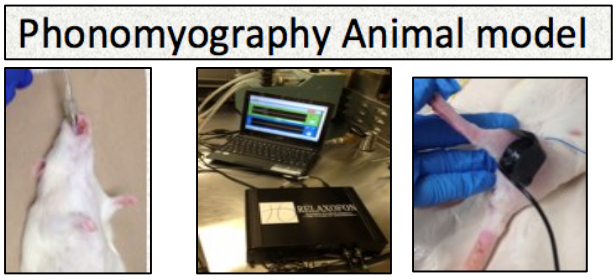


FIGURE 10. Left: animal intubation. Middle: phonomyography system. Right: positioning of microphone for phonomyography.

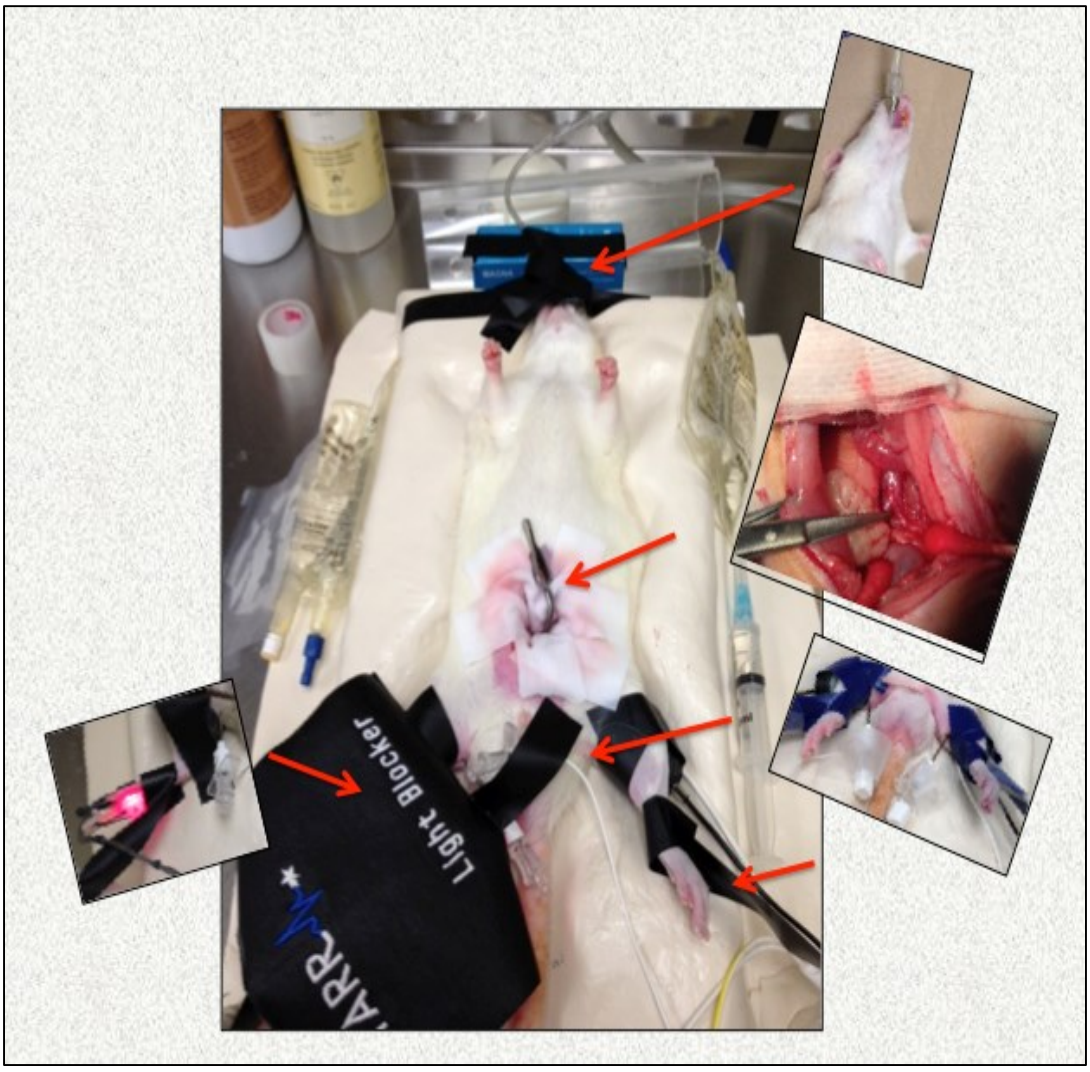


FIGURE 11: PROCEDURE ISCHEMIC MODEL

Picture of anesthetized, intubated rat in supine position. A laparotomy provides access to the left common iliac artery for reversible clamping. A saturation probe confirms absence of perfusion in the left foot upon clamping and is then transferred to right foot to provide intraoperative cardiac monitoring. A percutaneous nerve stimulator is positioned next to the sciatic nerve in both thighs. A phonomyography microphone is taped to the calf on both sides to monitor acoustic output upon contraction.

5.2 Harvesting muscles and nerves

On the fourth post-operative day (Nouri et al (52) proved maximal ischemic changes at the 4th day post injury), the rats underwent a second general anesthesia to allow harvesting of muscle (tibialis anterior, extensor digitalis longus) and nerve (common peroneal and tibial) from the right and left leg according to a standard protocol (maintaining tension on muscle for processing with sarcomeres out to length). Muscles and nerves were harvested with a rigorous technique to maintain muscle length and intact nerves for the analysis. As Lawendy and Sanders (44) proved in they study the pressures evens in all the compartments of the limb of the rat after infusing normal saline in only one compartment, this is why we decided to infuse in only one compartment. Muscle and nerve specimens where preserved in formalin (10% neutral buffered formalin, Fisher, cat 23-245684) and labelled with an alpha-numeric system to ensure blinded analysis by the pathologist.

Light microscopy

The muscles and nerves where harvested, processed and embedded in paraffin, for light microscopy, and stained with haematoxylin and eosin. The samples were analyzed by a blinded expert veterinary pathologist. Three sections in row from each specimen and three random fields at high power field (HPF) level from each section were chosen and the results were averaged. An objective grading scale was used for the histological analysis (52). The muscles where graded as normal if no ischemic changes where noticed or a percentage was given if ischemic changes where present such as: Segmental degeneration, necrosis and regeneration (graded in percentage). Grading for edema was based on severity and distribution. The nerves were graded 0 to 4: depending of amount of ischemic changes present such as: distension and fragmentation of myelin sheaths multifocally, axonal swelling/ degeneration. Graded: 1: modest/rare, 2: mild/infrequent, 3: moderate/frequent, 4: severe/extensive, the grading corresponded to percentage of fiber

ischemia and edema throughout the sections <2%, 3–25%, 26–50%, 51–75%, and >75%, respectively. See table 2.

Table 2. **Histopathology grading of ischemia and edema**. Adapted from Nouri et al (52).

Histopathology grading of ischemia and edema	
Muscles Grading	**Nerves Grading**
N: normal (No ischemic changes present)	N: normal (<2%)
1-100% Percentage of muscle damage	1: modest/rare (3-25%) 2: mild/infrequent (26-50%) 3: moderate/frequent (51-75%) 4: severe/extensive (>75%)
Ischemic changes that where present: Segmental degeneration, necrosis and regeneration.	Ischemic fiber degeneration that was present: severity and distribution of edema, distension and fragmentation of myelin sheaths multifocally, axonal swelling/ degeneration.

Chapter 6 Experiments: learning curve

This project was technically demanding on many levels and required learning across many areas including basic rat anatomy and physiology, different types of materials used and specific circumstances (explained below) that made the experiments more reliable and replicable.

1) Anesthesia and intraoperative support: We started by ventilating the animals with the use of a mask; however, we realized that for a prolonged operative procedure lasting up to 6 hours, the animals needed more sophisticated ventilatory support than a mask provided. We thus instituted endotracheal intubation and, in combination with more aggressive body temperature maintenance, a zero percent intraoperative mortality rate was achieved.

2) Positioning of rat during the experiment. Early in our experimental experience, we observed that maintenance of a stable, comfortable and secure position was critical to success. Any shift of the animal's position could dislodge the intubation equipment. The nerve stimulators and microphones were also highly sensitive to position and any change would alter the signal quality and intensity. In order to secure a stable and safe position, we experimented with different thermoplastic materials to develop an "operating bed" for the rat. The development of this supportive surface ensured a safe environment for the animal as well as consistent and reproducible phonomyographic results.

3) Level of arterial occlusion: Based on prior ischemia models in the rat (ref), we initially clamped the left femoral artery to render the lower extremity ischemic. However, initial phonomyography and histology results suggested that we had not achieved complete interruption of blood flow, likely due to collateral circulation proximal to the site of occlusion. Further research on the topic revealed other studies that described clamping of the left common iliac artery as an effective way to induce ischemia of the lower limb in

the rat (52, 90, 91). Through this method we obtained complete ischemia of the limb confirmed by pulse-oxymetry (MouseOX) and pallor of the extremity.

4) Reliable nerve stimulation. Initially we attempted nerve stimulation using a transcutaneous technique. Unfortunately, this technique was not reliable, as the electrode would detach from the skin, thus affecting the quality of the nerve stimulation. With the assistance of different anesthesiologists at our institution, we learned that the most reliable nerve stimulation technique for our project was with percutaneous nerve stimulation. Percutaneous nerve stimulation of the sciatic nerve in the posterior thigh, using the motor response of the posterior calf muscles as a guide, permitted accurate and dependable nerve stimulation throughout the experiment.

Chapter 7 Histological analysis

Histological analysis to establish the degree of muscle and nerve ischemic damage as a result of varying ischemia times was performed by an expert veterinary pathologist who was blinded to the duration periods. The right extremity biopsies, also a part of the blinding process, were used as a control. Grading of muscle and nerve damage was performed according to validated published grading systems (52).

Two groups of skeletal muscles were submitted: tibialis anterior muscle and extensor digitorum longus (EDL) muscle. The right limb served as a control and the left one as ischemic model, thus giving 4 muscle samples per animal: 2 ischemic and 2 controls This allowed for direct comparison between the histopathological changes found on the ischemic and control limb. A total of 15 animals underwent ischemia, giving 60 muscle samples that were analysed. The extent of the ischemic injury of the tissues was graded according to the amount of injury seen on the pathology slide and it was graded in percentages.

Two groups of peripheral nerves were submitted: common peroneal nerve (fibular nerve) and tibial nerve. There were 4 nerve samples per animal, 2 from the control limb and 2 from the ischemic limb. A total of 15 animals underwent ischemia, giving 60 nerve samples that were analysed. Several of the nerves had distension and fragmentation of myelin sheaths multifocally with axonal swelling/degeneration. These pathological changes were graded semi-quantitatively; grade 1=modest, rare; grade 2=mild, infrequent; grade 3=moderate, frequent; grade 4=severe, extensive. Nerves and muscles were analysed in detail. Different levels of injury were found such as: axonal swelling and degeneration of Schwan cells. Muscle injury included muscle segmental degeneration, necrosis, regeneration, necrotic myosites, coagulative necrosis associated with ischemic results, infiltrated by macrophages and satellite cells (stem cells that regenerate muscle) cells with nuclei centrally localized representing myosites regenerating.

Chapter 8 Results

8.1 Results of Histological analysis

Histological analysis of muscles and nerves harvested was done for all the 15 rats while taking into consideration the ischemic time for the analysis of results. There were 5 different ischemic times: 30min, 1hour, 2 hours, 4 hours and 6 hours. For each of the ischemic times, muscles and nerves were harvested from the control and ischemic limb of each animal. See figures 13 and 14 for details of specific histology changes and the corresponding slide. A grand total of 120 slides were analyzed, 60 muscles and 60 nerves harvested for all 15 animals. Figures 12, 13.

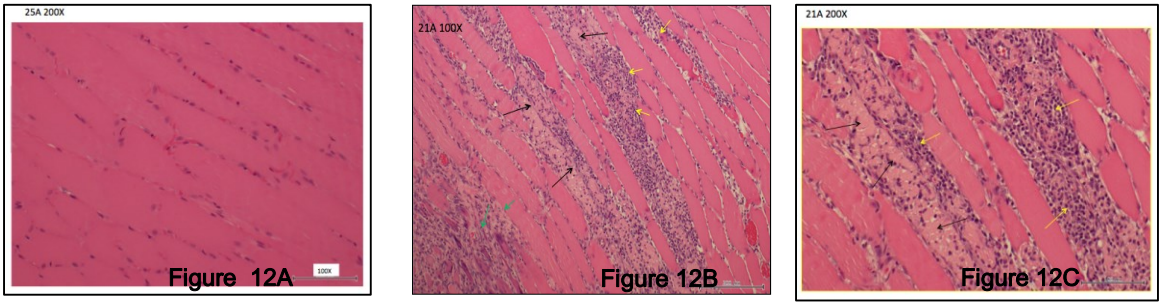
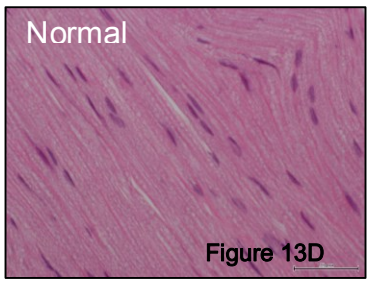
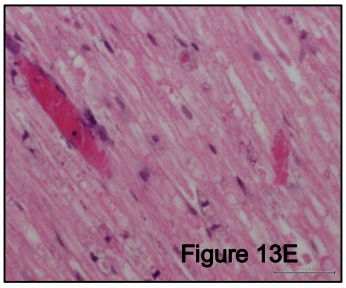
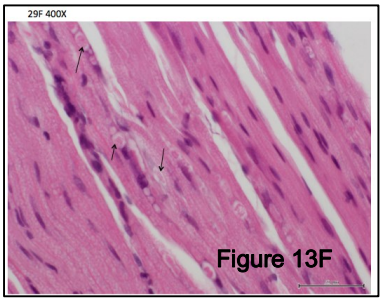
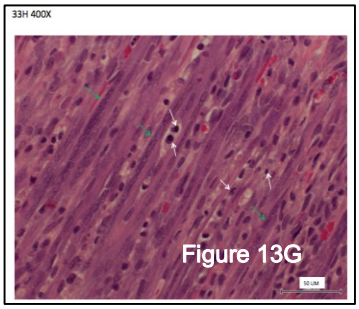


FIGURE 12 (A,B,C): Histology: Haematoxylin & eosin stain under light microscopy. A:Tibialis anterior muscle control, normal muscle (25A). B:Tibialis anterior control 1h ischemia 100X magnification, with 5% degeneration.(21A). Black arrows: necrotic myocytes undergoing floccular degeneration. Yellow arrows: macrophages and satellite cells are infiltrating the necrotic muscles. Green arrows: damaged myocytes are regenerating. C : Tibialis anterior Control 1h ischemia 200X (21A).









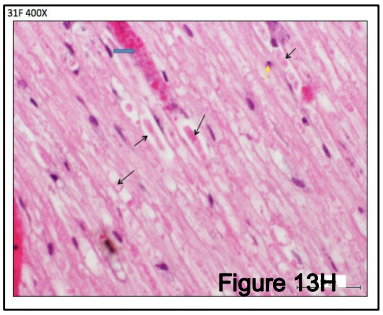


FIGURE 13 : D,E,F,G,H HISTOLOGY
D: Normal Tibial Nerve from control limb.
E: Tibial Nerve Ischemia 6h. 400x.
F: Tibial Nerve Ischemia 4h, 400x (29F). Black arrows: axonal swelling/degeneration with fragmentation of myelin.
G: EDL (Extensor digitorum longus muscle) EDL Ischemia 6h. 400x. The pathological changes here are farther in the healing process when compared with the 1h ischemia. Green arrows: Regeneration myofibers. The myofibers have a small diameter and a central rowing of nuclei. White arrows: apoptotic cells (likely inflammatory cells in apoptosis). It is part of the cleaning process.
H: Tibial Nerve Ischemia 6h. 400x (31F). The pathology changes are more extensive in this picture than they are in 4h of ischemia. Black arrows: axonal swelling/degeneration with fragmentation of myelin. Large blue arrow: damaged vessel with peripheral hemorrhage. Yellow arrow: apoptotic cell.

Each of the ischemic times were analysed. There were 3 animals per ischemic time: a, b and c. The results of ischemia found on each time point of ischemia are found in Table 3. For each ischemia time, there was some degree of ischemia in muscles and nerve. We found more ischemic changes and tissue injury as the ischemic time increased, observing only minimal changes (1%) in two samples of the 30-minute ischemic time. The histologic

changes where more evident as the ischemic time increased. At 1hour of ischemia, there was up to 10% muscle and nerve damage. At 2 hours of ischemia, there was 100% tissue damage. At 6 hours of ischemia, there was 100% tissue damage in 2 rats whereas the third rat demonstrated minimal decrease in phonomyographic signal and no evidence tissue necrosis, suggesting occlusion of the iliac artery was not achieved.

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RESULTS HISTOLOGY 30 MIN

Mean % decrease signal ISCHEMIA	21	44	44

MUSCLES	30min (a)	30min (b)	30min (c)
Control	N Tib ant N: EDL	N Tib ant N: EDL	N: Tib ant N: EDL
Ischemia	1% Tib ant N: EDL	N: Tib Ant N: EDL	N: Tib Ant N: EDL

NERVES	30min (a)	30min (b)	30min (c)
Control	N: CPN N: Tib N	N: CPN N: Tib N	N: CPN N: Tib N
Ischemia	N: CPN N: Tib N	1+ CPN N: Tib N	N: CPN QIA: Tib N

Histopathology 1h

Mean % decrease signal CONTROL	9	18	17
Mean % decrease signal ISCHEMIA	21	44	44

Muscles	1h (a)	1h (b)	1h (c)
Control	N Tib ant N: EDL	N Tib ant N: EDL	5%: Tib ant N: EDL
Ischemia	10% Tib ant N: EDL	N: Tib Ant N: EDL	10%: Tib Ant N: EDL

Nerves	1h (a)	1h (b)	1h (c)
Control	N Tib ant N: EDL	N Tib ant N: EDL	5%: Tib ant N: EDL
Ischemia	10% Tib ant N: EDL	N: Tib Ant N: EDL	10%: Tib Ant N: EDL

Histopathology 2h

Mean % decrease signal CONTROL	14	38	8
Mean % decrease signal ISCHEMIA	46	76	72

Muscles	2h (a)	2h (b)	2h (c)
Control	N Tib ant N: EDL	1%: Tib ant N: EDL	N: Tib ant N: EDL
Ischemia	N: Tib ant N: EDL	80% Tib Ant 100% EDL	5%: Tib Ant N: EDL

Nerves	2h (a)	2h (b)	2h (c)
Control	N: Fib N QIA: Tib N	N: Fib N 1+ Tib N	N: Fib N N: Tib N
Ischemia	N: Fib N N: Tib N	N: Fib N N: Tib N	N: Fib N QIA: Tib N

Histopathology 4h

Mean % decrease signal CONTROL	8	12	27
Mean % decrease signal ISCHEMIA	50	46	86

Muscles	4h (a)	4h (b)	4h (c)
Control	N Tib ant N: EDL	N Tib ant N: EDL	1% Tib ant N: EDL
Ischemia	75% Tib ant 50% EDL	10% Tib Ant N: EDL	90% Tib Ant 100% EDL

Nerves	4h (a)	4h (b)	4h (c)
Control	N: Fib N N: Tib N	N: Fib N N: Tib N	N: Fib N N: Tib N
Ischemia	QIA: Fib N N: Tib N	QIA: Fib N 1+ Tib N	4+ Fib N 2+ Tib N

RESULTS HISTOLOGY 6h

Mean % decrease signal ISCHEMIA	33	95	66

Muscles	6h (a)	6h (b)	6h (c)
Control	N Tib ant N: EDL	N Tib ant N: EDL	N Tib ant N: EDL
Ischemia	N: Tib ant N: EDL	100% Tib Ant 100%: EDL	85% Tib Ant 100% EDL

Nerves	6h (a)	6h (b)	6h (c)
Control	N: Fib N N: Tib N	1+ Fib N N: Tib N	N: Fib N N: Tib N
Ischemia	N: Fib N N: Tib N	2+ Fib N 4+ Tib N	N: Fib N 1+ Tib N

TABLE 3

TABLE 3: HISTOLOGY RESULTS. Phonomyographic and histologic results of all time points of ischemia 30min, 1h, 2h, 4h and 6h. N=normal tissue, 1-100% represents percentage of muscle ischemic changes, QIA: insufficient sample quantity for analysis, 1-4 grading of nerve damage

8.2 Results of Phonomyography signal

The data was analyzed with Wilcoxon signed rank test, for non-parametric paired data, a CI (Confidence interval) of 95% was used. The date of each of the 3 rats was compared and the difference between the control and the ischemic limb was obtained. At the same time the signal was compared in the same animal to see the change of the phonomyographic signal compared with the ischemic time. Changes of the phonomyographic signal over the ischemic time where calculated and represented in mean (Table 4). The phonomyography showed a significant decrease in the low frequency signal output from injured muscle, which corresponds with the duration of muscle and nerve injury and histologic finding in the ischemic model (Tables 5-11). At all-time points of ischemia, there was a statistically significant decrease of the phonomyography signal except for 6 hours of ischemia. At 30min of ischemia, the signal decreased up to 45% (n=15; p=0.005), corresponding with rare (1+) nerve damage and 1% muscle necrosis. At 1h of ischemia, the signal decreased up to 55% (n=12; p=0.005), corresponding to 5-10% of muscle necrosis. At 2h of ischemia, the signal decreased up to 76% (n=9; p=0.015), corresponding to 100% of muscle necrosis and rare (1+) nerve damage. At 4h of ischemia, the signal decreased up to 86% (n=6; p=0.028), corresponding to up to 100% of muscle necrosis and severe (4+) nerve damage). At 6h of ischemia, there was a decrease of phonomyography signal up to 95% (n=3; p:0.109), corresponding to 100% of muscle damage and severe (4+) nerve distension and fragmentation of myelin sheaths multifocally with axonal swelling/ degeneration.

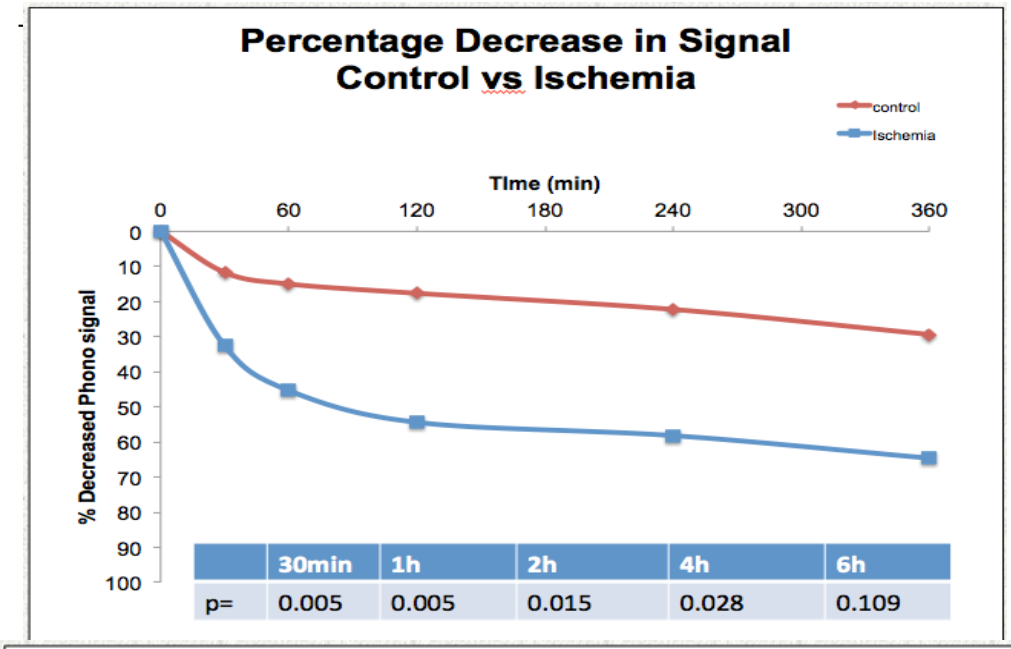
Table 4. Change in the phonomyographic signal over time of ischemia (Mean)

	95%CI	30 min	1h	2h	4h	6h
Ischemia	Lower limit	22.0	31.3	34.8	35.8	29.5
	Mean	32.6	45.3	54.4	58.2	64.6
	upper limit	43.2	59.3	74.0	80.6	99.7

	95%CI	30min	1h	2h	4h	6h
Control	Lower limit	6.9	8.5	8.5	7.9	4.7
	Mean	11.7	15.0	17.6	22.2	29.4
	Upper limit	16.5	21.5	26.8	36.5	54.0

At all ischemic times, the signals were compared between the ischemic and control limbs for all the animals that underwent the prescribed time of ischemia. For example, in the 30 min ischemia, the signal was analyzed for all the animals that underwent the same or more than 30min of ischemia. Therefore, the total number of signals analyzed for 30 min of ischemia was 15. For the 1h ischemic group, the total number of signals analyzed was 12 since the vascular clamp was released in 3 rats at 30 minutes. Consequently, the number of signals analyzed in each group decreased by an additional 3 rats.

A statistically significant decrease in phonomyography signal between ischemic and control limbs was observed for all groups except the 6-hour group. The 3 animals used for 6 hours of ischemia showed no statistical significance most likely due to the small sample size, despite a 100% muscle and nerve damage in the two animals that obtain complete limb ischemia.



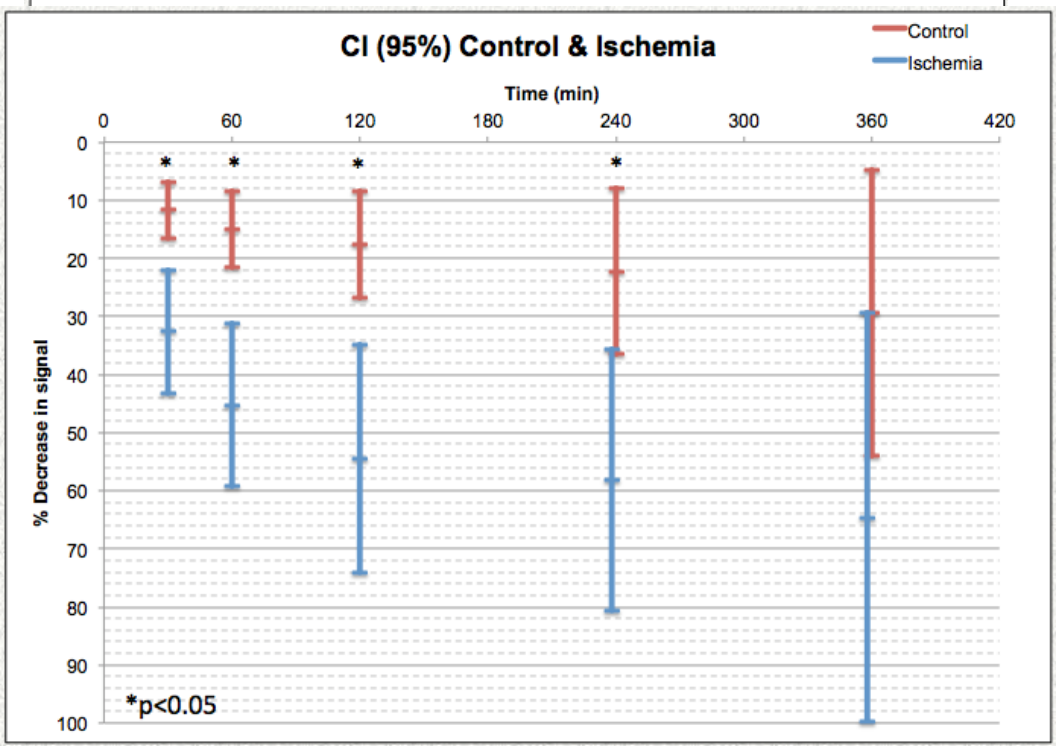


TABLE 6

TABLE 5-6: Summary of mean phonomyographic output decrease in ischemic (blue) and control (red) limbs over time with 95% confidence intervals and p-values.

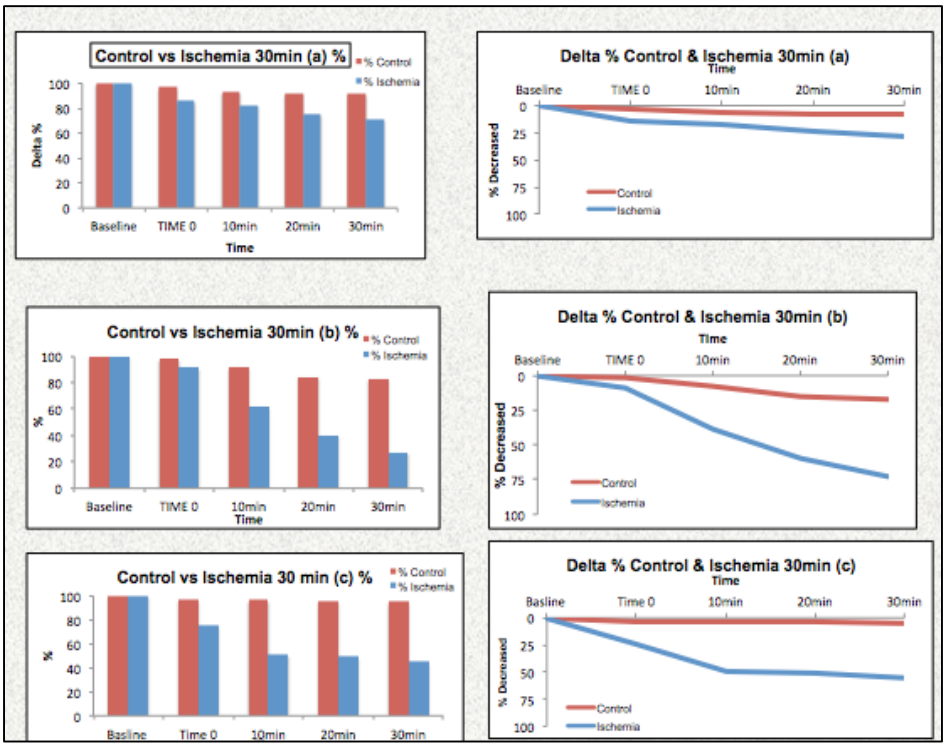


TABLE 7:

TABLE 7: phonomyography signal results 30 minutes

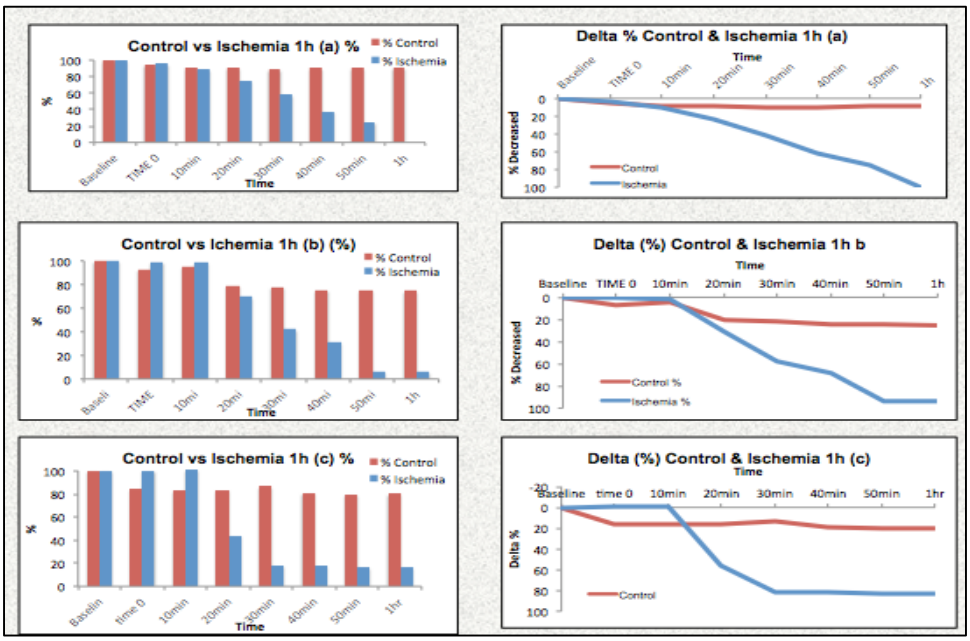


TABLE 8

TABLE 8: phonomyography signal results 1 hour

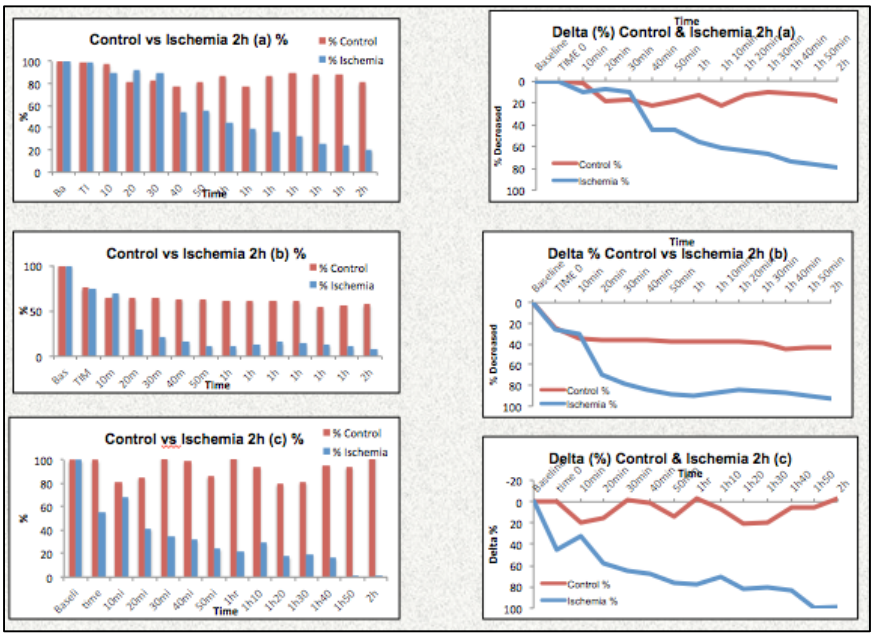


TABLE 9

TABLE 9: phonomyography signal results 2 hours

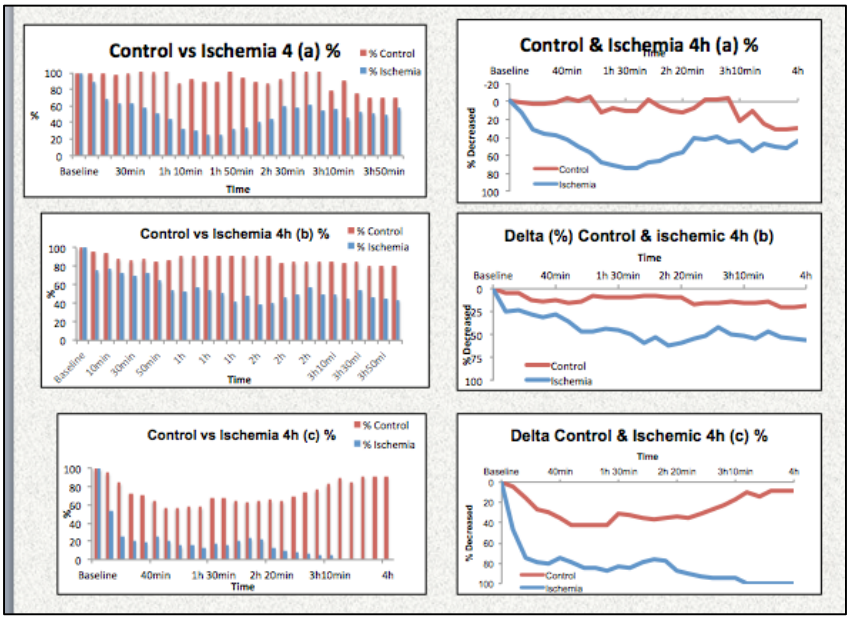


TABLE 10

TABLE 10: phonomyography signal results 4 hours

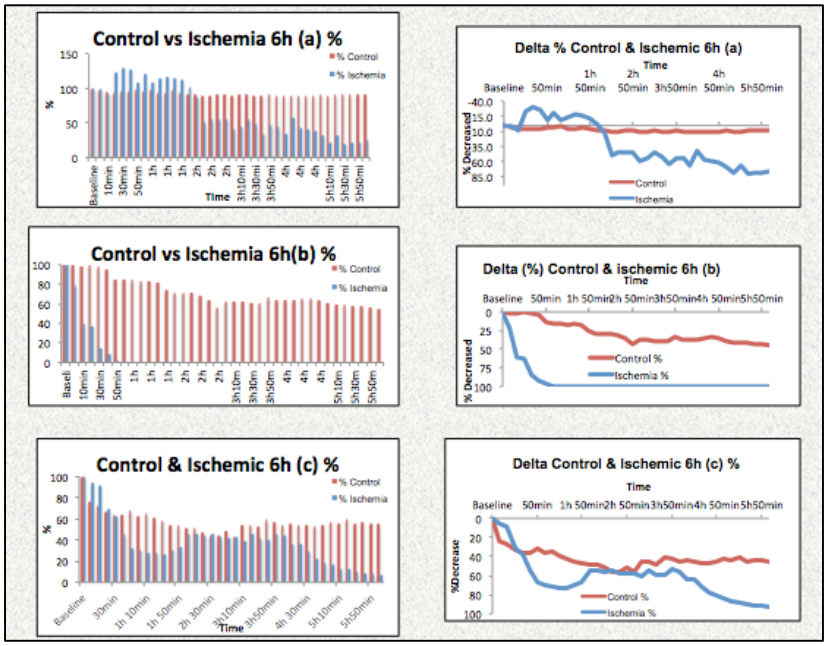


TABLE 11

TABLE 11: phonomyography signal results 6 hours

Chapter 9 Discussion

The purpose of this project was to establish phonomyography as a potential non-invasive continuous monitoring technique to diagnose ACS in an injured limb. Phonomyography's clinical role to date has focused on the study of neuromuscular blockade in anaesthetized patients (92-94). We sought to test the phonomyographic response of ischemic muscle in a murine animal model in order to support our hypothesis that this acoustic detection modality could serve as novel detection method for a limb-threatening condition, acute compartment syndrome. Although further research is required, the acoustic and histologic results of this study are promising and support our hypothesis that phonomyography is a reliable tool to continuously monitor ongoing limb ischemia.

Multiple diagnostic methods to detect ACS exist. However, there are none that can reliably diagnose this condition without an invasive technique such as intra-compartmental pressure measurement with an indwelling needle. Thus, clinicians still rely on clinical symptoms in the awake patient, while they must rely on invasive techniques in the unconscious, obtunded, or insensate individual. The development of a non-invasive continuous monitoring technique would significantly improve our ability to diagnose ACS in a timely manner and prevent the devastating consequences of a delayed or missed diagnosis (95).

The aim of early diagnosis of ACS is to limit potential functional disabilities due to irreversible ischemic injury to nerves and muscles in the affected limb segment. Although patient well-being is the primary concern, there are also additional important medico-legal aspects that should be taken into consideration. Bhattaharyya et al(96) reported that it is not only important to avoid the consequences and sequelae from ACS, but also to decrease the risk of malpractice. They reviewed medical records with claims of ACS and malpractice between 1980 and 2003. A liability of $3.8 million dollars per case was paid as compensation in the cases settled in the favour of the plaintiff. "Increasing time from the onset of symptoms to the fasciotomy was linearly associated with an increase

indemnity payment. A fasciotomy performed within eight hours after the first presentation of symptoms was uniformly associated with a successful defense." They emphasize the importance of early diagnosis and fasciotomy to improve patient outcome and decrease indemnity risk.

Vaillancourt et al(97) described a study of muscle necrosis in a cohort patients who underwent fasciotomy for ACS in 4 large teaching hospitals. They noticed that even after 3 hours of ACS there was evidence of muscle necrosis. This study noted that 82% of ACS cases presented following a traumatic incident and that 49% of ACS patients had muscle necrosis proven by pathology. Of these patients, 30% lost up to 25% of the muscle mass in the affected compartment. Surprisingly, muscle necrosis was present in 2 out of 4 patients who underwent surgical decompression within 3 hours of the injury, thus the importance of early diagnosis and surgical intervention. They concluded in a survival analysis that 37% of all cases of ACS might develop muscle necrosis within 3 hours of injury.

Given that the muscle and nerve necrosis that occurs in ACS is secondary to ischemia provoked by collapse of the microvascular components of the circulatory system (capillaries, venules) from the increased intra-compartmental pressure, it was pertinent to evaluate the performance of phonomyography in a purely ischemic model as a first step. Challenges to establishing a reliable ischemic model have been outlined above. However, once these obstacles were overcome, this study model provided robust data to support our hypothesis.

We were able to correlate a decrease of the phonomyographic signal with the duration of tissue ischemia as well as with an increase in tissue injury as determined by histology. Our data demonstrated a statistically significant decrease in the phonomyography signal as early as 30 minutes following the start of ischemia.

The histological results of the rats exposed to 6 hours of ischemia revealed that 2 out of 3 rats had 100% muscle and nerve damage but one rat had normal histology.

Interestingly, this animal's phonomyographic signal remained similar to the control (perfused) limb, suggesting that despite what appeared to be successful clamping of the common iliac artery and loss of the pulse using pulse-oximetry, this limb remained normally perfused. Possible explanations include anomalous collateral circulation or possible slip of the vascular clamp allowing blood flow to the leg. This may have further contributed to the lack of statistical difference in phonomyographic signal between ischemic and control limb in the 6-hour group resulting in a false negative finding. There was no statistical difference at 6 hours of ischemia most likely due to a small sample size. Even though it is unlikely we must also consider that the 6 hours of ischemia giving no statistical difference could be a true result (this could be tested in further studies with bigger sample size). Given that 3 animals were assigned to each duration of ischemia, there was a progressive decrease in the sample size by 3 rats at each time interval. For example, all 15 animals endured 30 minutes of ischemia, 12 were exposed to ischemia for 60 minutes while only 3 had a full 6 hours of ischemia. We posit that the difference in acoustic output between the ischemic and the normally perfused legs would have remained statistically significant had we had more specimens at 6 hours.

In terms of histologic evidence of tissue damage and association with phonomyographic output, in our experiments we were able to show changes in the phonomyography signal as time of ischemia increased and evidence of ischemic changes in the limb proven histologically. Our results showed that at 4 and 6 hours of ischemia, muscles and nerves in the affected compartments had suffered significant and irreversible damage. This is in keeping with previous reports (44, 52, 55, 91, 98). For example, these findings are similar to those of Nouri et al 2008 (52), in which they used an animal model with Sprague Dawley rats (the same animal model that we used) to show that following 4 hours of limb ischemia, there was presence of axonal swelling and progressive nerve damage, as confirmed by light microscopy. In detail Nouri et al (52) mentioned as results after reversible limb ischemia: after 4h of ischemia: axonal swelling, after day 1: epineural edema, after day 4 and 7: marked endoneural edema; after 7 days: ischemic fiber degeneration and axonal swelling of sciatic nerve, severe edema. Our histological analysis also confirmed the

presence of axonal swelling and degeneration with fragmentation of myelin at 4 hours of ischemia. After 6 hours of ischemia, in addition to the above damage, there was also evidence of regeneration in the histological analysis, with myocytes demonstrating central rowing of nuclei and apoptotic cells. Lawendy et al (44) found similar results in an animal ACS model, noting an inflammatory response with activated leucocytes, and decreased vascular supply that increases the cellular injury and the inflammation of the tissue.

In conclusion, this study has supported the hypothesis that ischemic muscle has a decreased phonomyographic output upon contraction when compared to normally perfused muscle. In addition, this decrease in sound production (phonomyography) becomes more pronounced as the duration of ischemia increases. Thus, phonomyography has potential as a noninvasive continuous monitoring technique for the detection of acute compartment syndrome. Further investigation will include testing this modality using an acute compartment syndrome animal model.

Chapter 10 Limitations

This project was done with no previous experience or studies of phonomyography in the diagnosis in ACS or in an animal model. There was a steep learning curve (see above Chapter 6: Learning Curve), regarding intubation of the animal, surgical procedure and monitoring of vital signs among others. Additional resources were required to complete the project within our proposed timeline. Some animals where lost as the experiments where taking too much operating time, requiring long time of sedation, we noticed that anaesthesia through a mask and oxygen was not enough. The animals required endotracheal intubation and close monitoring of vital signs as well as maintenance of proper body temperature all these changes as well as optimization of the surgical technique and practice throughout the hole procedure decreased the rats mortality to cero. Some animals were lost in the early phase of our study, which further decreased

our sample size. Despite conclusive histology findings at 4 and 6 hours, microscopic electron microscopy was not performed due to time constraints and limitation in funding. Given that no previous reports exist in the literature assessing phonomyography for diagnosing ACS, a direct comparison of our results with other studies cannot be made.

Percutaneous nerve stimulation was performed in our animal model due to different factors that are not necessarily present in humans, such as elasticity of the skin that dislodges or displaces the nerve stimulator particular in rats, very small nerves and small lower extremities. In humans, transcutaneous instead of percutaneous nerve stimulation can be done in a reliable fashion, avoiding breaking the skin barrier.

Chapter 11 Conclusions

An ischemic animal model of ACS was studied showing muscle and nerve damage of the limb. Phonomyography was used for the first time demonstrating a change in acoustic signal that correlates with ischemic time. Histological analysis was performed to correlate the degree of muscle and nerve damage with specific time of ischemia.

A steep learning curve allowed for application of the phonomyography in the detection of neuromuscular ischemic lesion in an ischemia and ACS animal model.

Phonomyography is a promising non-invasive technique to detect early changes in muscle physiology that occur as a result of acute compartment syndrome. This was observed by showing alterations in the acoustic signal emitted by the muscles of an injured/ischemic limb.

Phonomyography reliably reflects muscle dysfunction induced by acute ischemia. Further testing is planned to evaluate its potential use in humans. Such studies will focus on the investigation of phonomyography signal in limb injuries and its potential application in the early diagnosis of ACS in trauma patients.

Chapter 12 ADVANCES

As part of this initial project, other ongoing experiments in ACS described below have been performed and final results are pending.

12.1 Acute compartment syndrome model: Normal Saline infusion

Using our previous ischemic model as a template and learning from pilot studies, we developed an ACS model where normal saline was infused in the limb to increase the compartmental pressure, with the aim to see difference of the phonomyography signal and histologic changes. The base of the experiments was exactly and replicated from the ischemic model of ACS, looking for phonomyographic and histologic changes that could we analysed in the normal saline infusion model and compare with the ischemic ACS model.

While following previously published studies that described an ACS model in animal limbs, normal saline was injected in the anterior compartment of the lower leg of the animal.(44) The blood pressure of the rat was taken in our initial models by catheterizing the carotid artery of the animal (44) However, due to the complexity of the procedure and high mortality, it was decided after several pilot models to measure the blood pressure (BP) with a tail cuff.

Explanation of the experiments and model

Based on replicating the ischemic ACS model that we did initially, the same number of animals where used as for the ischemic model for ACS. Fifteen Sprague Dawley rats where used and infusion of normal saline was performed in the anterior compartment of the lower extremity to increase the pressure. The animals underwent acclimatization as per protocol (see above description for ischemic ACS model). The experiments where performed with the animal under anesthesia and endotracheal intubation. Proper hydration, analgesia as per protocol (Carprofen see above), temperature control and vital signs monitoring where performed throughout the experiments. Once normal saline was infused in the anterior compartment of the limb (see below explanation); the pressure was

constantly monitored and maintain through the prescribed time of ACS model. Just like in the ischemic ACS model the time with increased pressure within the compartment caused by the infusion of normal saline was: 30 minutes, 1, 2, 4 and 6 hours. The right limb serve as control and the left as experiment where the normal saline was infused. Both extremities where stimulated with percutaneous nerve stimulation every 10 minutes. The phonomyography microphone was applied in the posterior aspect of the limb and the acoustic signal was recorded in both limbs (exactly as descried in the ischemic ACS model, see above).

Infusion of Normal Saline to create ACS in the limb and Experiments:

The animal was positioned supine on the "operating bed" created specifically for accuracy of tests performed (see ischemic ACS model explanation), the legs were fixed in the "operating bed" and the microphones where fixed on the limb. Tail pressure cuff was applied to record the BP.

As per previous studies by Lawendy and Sanders (44), infusion of normal saline in the anterior compartment of the animal limb and maintenance of this pressure for 30-45 minutes resulted in equal pressure in all the compartments. Once the rats were anesthetized, we elevated compartment pressure by slowly infusing isotonic normal saline via a 24-gauge angiocatheter into the anterior compartment of the left hind limb in the experimental group. Compartment pressure was raised to 30 mm Hg and maintained between 30 and 40 mm Hg for the duration of the protocol. A second syringe of 14 gauge was inserted in the same anterior compartment, but as distal as possible from the infusion syringe, to measure with a monitor and an arterial line the intra-compartmental pressure (Monitor 530.411). The pressure measured with the monitor was compared with the Compartmental Pressure Monitoring System *(DePuy-Synthes)* for continuous measurement of intracompartmental pressure and the readings where always the same in both the Synthes monitor and the Arterial line monitor. The elevated pressure in the compartments was maintained for the prescribed period of ischemia: 30minutes, 1 hour, 2 hours, 4 hours and 6 hours.

Following the defined period of normal saline infusion ACS, fasciotomy was performed to eliminate the increase of pressure in all the compartments of the limb. The animals where then let to recover, and appropriate postoperative analgesia was provided according to SOP 101.02 (Rodent analgesia) same protocol used in the ischemic ACS model. Adequate postoperative care, including dressing changes, feeding, analgesia and hygiene where done for 4 days as it was done in the ischemic ACS model due to previous studies showing maximal nerve and muscle damage at 4 days(52). After 4 days, muscles and nerves where harvested and the animal was sacrificed. As per the above mentioned protocol for the ischemic model of ACS the harvested tissue was: tibialis anterior muscles, extensor digitorum longus muscles from the control and ACS limbs and the common peroneus and tibialis nerve form the control and ACS limbs. Standard histopathological, light microscopy Haematoxylin and eosin analysis was performed to detect and quantitate ischemic changes, as in the ischemic model (pending histology results and analysis of data). See Figure 14 for explanation of settings for experiments. Correlation will be made between duration of normal saline infusion model of ACS, the degree of muscle necrosis, and the phonomyographic output.

Settings of normal saline infusion, ACS model.

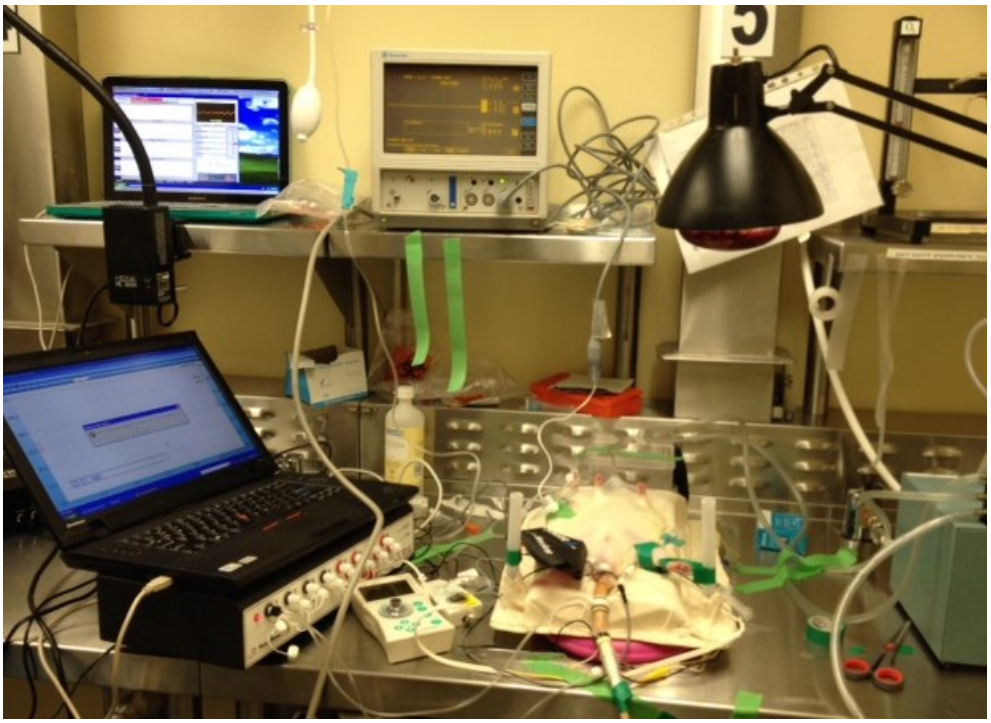


FIGURE 14

The rat is anesthetized, endotracheal intubation is preformed, and the animal is positioned on the “operating table”. Monitors are needed to visualize and monitor vital signs, blood pressure (notice tail BP cuff), a monitor and arterial line is used to monitor the infusion of normal saline and the corresponding increase of intra-compartmental pressures. Left limb has needles infusing normal saline and measuring the compartment. Phonomyography microphones are positioned under the thigh of the control and ischemic limbs. Heating lamp and heating pads are used (pink pads under the “operating table), to maintain proper temperature of the animal throughout the procedure.

Discussion:

The decision of using the tail blood pressure of the animal to monitor and create the normal saline model of ACS in the limb was done due to the high mortality of the animals during the cannulation of the carotid artery which was describe in the stud of Lawendy(44). Also, it was noticed that keeping the animal alive with the catheter in the

artery increased the morbidity and complications of the procedure, making it very difficult to replicate the procedure in a reliable fashion with other animals. Taking all these into consideration the decision of using the tail blood pressure measure was done knowing that less animals where lost for the purpose of the study, that was proving that this was a valid model. Lawendy(44) cannulated the carotid artery and kept the animal alive with ACS for 45min, in our experiments that aimed to see muscle and nerve damage in different ischemic periods caused by ACS, part of the animals needed to maintain the ACS and a high compartment pressure for more than 45minutes, as we tested up to 6 hours of ACS. We also kept the animals alive for four days post intervention. During the four days after the ACS was created the animal needed to be kept in the best circumstances with pain control and no exposed wounds, this is why a fasciotomy was done and the animal was monitored on daily bases until the day four, when the muscles and nerves where harvested.

These experiments where normal saline was injected in the limb of the murine animal to create an ACS are preliminary and where based completely on the experience and all that was learned in the ischemic ACS model. Further analysis of histology and phonomyography results are pending and will be continued not as part of this thesis but as part of the common and genuine interest of these research group and the amazing motivation of the supervisor.

APPENDICES

PROTOCOL:

PHONOMYOGRAPHY AND ISCHEMIC MODEL

Materials:

- Sprague Dawley rats 250-350gr
- OR facilities
- Isoflurane
- O2
- Anesthesia machine
- Equipment for ventilation with intubation
- Tube for endotracheal intubation with guide
- Bed for rats intraoperative for positioning and traction
- Scotch tape
- Gloves
- Face mask
- Surgical cap
- Warming light
- Warming blanket
- Normal saline
- Eye lubricant (provided by the lab)
- Q tips
- Surgical instruments: Blade, needle holder, scissors, pickups, retractor, suture (nylon 0, monocryl 2-0), microvascular clamps
- Chlorhexidine
- Iodine solution
- Gauze 2x2
- Syringe 5cc
- Syringe 10mm

- Carprofen: analgesia for rats (prepared by animal facility)
- Intubation box for positioning of the rat
- Rubber elastic bands
- Scissors
- Lubrigel, for intubation
- Shaver
- Forms for documentation of procedure, vitals and time of intervention (see form A)
- Form to document ID of rat and intervention performed with expected day for samples and sacrificing the animal. (See form B)

Label properly the rat that will be used ex: 30min ischemia= RAT #10, and decide a distinctive mark to identify the rat ex: one, two or three lines circular around the rats tail with permanent marker (this will stay up to 5 days).
Before starting make sure the anesthesia machine has full Isofluorane and that the O2 is running.

Anesthesia:

Induction:

- Holding the rat from the tail proximal to the lumbar spine, position the rat in the plastic box and close the cover, make sure the tubing is connected properly from the anesthesia machine to the plastic box with proper seal to avoid anesthetic leaking out of the box.
- Anesthesia induction: Start the O2 at 1Lt flow and the Isofluorane at 5%, leave the rat until it falls asleep.
- Once the rat is asleep, take out the rat by the tail and position in the plastic box on the scale, obtain weight of the animal and document it. (needed for calculation of analgesic, carprofen dose)
- Place the rat on the operating table and shave the lumbar area and para lumbar muscles area, shave both calves anterior and posterior.

- If the rat begins to awaken from the manipulation, place the rat back in the box and administer the same O2 and isofluorane as for induction.
- Prepare the ventilation machine, turn on and connect to the anesthesia machine. The ventilation machine SHOULD be connected to the bottom part of the anesthesia and O2 machine, make sure all the cables are well connected.
- Prepare the intubation table (triangle made of styrofoam) with an elastic band around it.
- Turn on light and shine on the apex of the intubation table
- Prepare lubrigel and apply small quantity to intubation tube.
- Intubation tube should have guide inside.

Intubation:

- Take the rat out of the box (used for induction)
- Place the rat supine on the induction table, with head hanging from the edge of the intubation table neck in hyperextension and light shining on the trachea.
- Place an elastic band inside the rat's mouth inferior to the superior incisors.
- Use a toothless pickup to pull on the base of the tongue.
- Visualize the vocal cords and insert the intubation tube.
- Holding the intubation tube in place and the rat remove gently the elastic cord from the mouth.
- Transfer the rat to the surgical table (table moulded and made with traction pols distally). DO NOT LET GO OF THE INTUBATION TUBE AND THE HEAD OF THE RAT as they easily get extubated.
- Place the rat supine on the surgical table, remove the guide from the intubation tube, connect the ventilation to the intubation tube, and secure the intubation and ventilation with tape to the rat and the table.

- Start the anesthesia of the anesthesia machine isofluorane 5% and O2 1Lt
- Increase the ventilation machine to respiration rate of 100, observe the chest of the rat. If the chest expands rapidly around 100x min as the ventilation machine then the rat was properly intubated.
- If the chest expands at a slower rate than 100/min of if the abdomen epigastrium starts to inflate, then the rat needs to be re-intubated.
- Once proper intubation is confirmed the Isofluorane can be decreased to 3%.

Maintenance anesthesia:

- isofluorane 2.5-3%
- During the surgical procedure or intervention the rat needs to maintain O2 at 1Lt.
- Must keep rat warm at all times: make sure the heating lamp is turned on and warms the rat
- Inject carprofen subcutaneous, under the skin of the chest laterally is best.
- Inject 2cc of NS for hydration
- Apply generous amount of eye lubricant to both eyes as rats do not close their eyes during anesthesia

Documentation: must be recorded on specific forms

- The time the anesthesia started
- The time of intubation
- Time of clamping of the artery
- Vital signs
- Any complication of difficulty present during the procedure

Dr. Adriana Martinez, Dr. Greg Berry 2015
PHONOMYOGRAPHY AND ACUTE COMPARTMENT SYNDROME MODEL
Form A

Label. ID:
Ischemia TIME: _____ DATE:_____.
Weight: -192 (Weight of rat container)= _____gr.

Anesthesia: START (time): _____ STOP(time):_____ .
Intubation (Time):_____ Extubation:
Carprofen (0.1ml/100gr):
Normal Saline (hydration): 2cc : Time_____,
Eye Ointment (time): _____.

Open abd (Time):_____ Closed abd (Time):_____.
Lt Common Iliac clamped: unclamped:
Complete recovery:_____ .
Nerve cath Stim @ mAmp:

TIME	ISCHEMIA	HR	O2 SAT	RR	ISO
First vitals after anesthesia					
	Clamp				
	10min				
	20min				
	30min				
	40min				
	50min				
	1h				
	1h10min				
	1h20min				
	1h30min				
	1h40min				
	1h50min				
	2h				
	2h10min				
	2h20min				
	2h30min				
	2h40min				
	2h50min				
	3h				
	3h10min				
	3h20min				

	3h30min				
	3h40min				
	3h50min				
	4h				
TIME	**ACS**	**HR**	**O2 SAT**	**RR**	**ISO**
	4h10min				
	4h20min				
	4h30min				
	4h40min				
	4h50min				
	5h				
	5h10min				
	5h20min				
	5h30min				
	5h40min				
	5h50min				
	6h				

Label. ID:
Ischemia X: _____ DATE:

NOTES:

HARVEST: DATE:
NOTES

Dr. Adriana Martinez, Dr. Greg Berry 2015
PHONOMYOGRAPHY AND ACUTE COMPARTMENT SYNDROME MODEL, FOR ANIMAL IDENTIFICATION.
Form B

No.	Ischemia time	Date: Sx	Date: Harvest	Label and ID
1	30min			
2	30min			
3	30min			
4	1h			
5	1h			
6	1h			
7	2h			
8	2h			
9	2h			
10	4h			
11	4h			
12	4h			
13	6h			
14	6h			
15	6h			

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