‘I saw the angel in the marble and carved until I set him free.’

- Michelangelo

University of Alberta

Investigation of Intermittent Electrical Stimulation as a Potential Prophylaxis against the Formation of Deep Pressure Ulcers after Spinal Cord Injury

by

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To my family.

Abstract

Deep tissue injury (DTI) is a *severe* form of pressure ulcers resulting from ischemia and mechanical damage due to unrelieved pressure. Despite many preventative methods, none so far has significantly reduced the incidence of DTI. The overall goal of my project was to investigate a novel method of pressure ulcer prevention, termed intermittent electrical stimulation (IES). The current study investigated the effects of IES on surface pressure and tissue oxygenation in individuals with SCI. The results demonstrated that IES-induced contractions caused significant reductions in pressure around the ischial tuberosities, as well as significant and sustained increases in oxygenation. Direct measurements of oxygen in an invasive rodent model indicated that IES-induced contractions resulted in a 20-100% increase in tissue oxygenation.

The results indicate that IES directly targets the pathogenic factors contributing to the development of pressure ulcers and thereby may be an effective method for the prevention of DTI.

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List of abbreviations

AB - able-bodied

ANOVA - analysis of variance

ATP - adenosine tri-phosphate

BOLD – blood oxygen level dependence

BW - body weight

Ca^{2+} - calcium

DNA - deoxyribonucleic acid

DTI - deep tissue injury

H^{+} - hydrogen ion

H_2O_2 - hydrogen peroxide

IES - intermittent electrical stimulation

ITs – ischial tuberosities

K^{+} - potassium

mA - milliamperes

MRI - magnetic resonance imaging

Na^{+} - sodium

NPUAP - National Pressure Ulcer Advisory Panel

NO – nitric oxide

O_2^{-} - superoxide

OH^{-} - hydroxyl radical

Pi - inorganic phosphate

PCr – creatine phosphate

SCI - spinal cord injury

SI – signal intensity

TcPO2 – transcutaneous partial pressure of oxygen

Chapter 1 – Introduction

1.1 Significance of Pressure Ulcers

Pressure ulcers are a significant concern for individuals with limited mobility and sensation [Cullum 1995], their caregivers and the health care system. Each pressure ulcer costs $15,000 to $73,000 to heal [Rischbieth 1998; Grip 1986]. The estimated annual cost of treating only those pressure ulcers that develop during a hospital stay is $2.2 to $3.6 billion [Zanca 2003] in North America and $2.6 to $4.0 billion in the UK [Bennett 2004]. Recently, the total cost of treating pressure ulcers in Canada was determined to be upwards of $3.5 billion [personal communication, Ferguson-Pell 2008]. Beyond the burden on health care resources, pressure ulcers further debilitate individuals with already compromised physical abilities, thereby reducing independence and lowering self-worth [Krouskop 1983]. This results in a considerable decrease in the quality of life [Spilsbury 2007].

The treatment of pressure ulcers can require lengthy hospital stays and drastic interventions, including surgical debridement and limb amputation. Preventative methods exist, such as repositioning and the use of specialized support surfaces, but none so far has provided a significant reduction in the incidence of pressure ulcers [Conine 1989; Krause 2004; Raghavan 2003; Salzberg 1996; Seymour 1985; Thomas 2003].

1.2 Significance of Pressure Ulcers in SCI

People at risk of developing pressure ulcers include nursing home residents, patients in hospital settings such as post-operative care and acute and critical care units, and those with neurological insults such as spinal cord injury (SCI) [Labbe 1987; Conine 1989; Woolsey 1991; Salzberg 1996; Zanca 2003; Edlich 2004]. People with SCI are particularly at risk due to their reduced sensation which lessens awareness of discomfort [Thiyagarajan 1984], and their limited mobility which presents challenges for repositioning to alleviate this discomfort.

1.2.1 Incidence and Prevalence in SCI

Despite comprehensive strategies by rehabilitation and public health professionals, prevalence rates of pressure ulcers in people with SCI are 30–60%, depending on the level and severity of the SCI [Richardson 1981; Krause 2001]. Moreover, annual incidence rates ranging from 20% to 31% [Byrne 1996; DeLisa 1986] have been reported and the recurrence rates can be as high as 91% [Niazi 1997]. Up to 80% of individuals with SCI develop pressure ulcers at least once in their lives [Salzberg 1996].

Wheelchair users are at risk of developing pressure ulcers in the tissue over the ischial tuberosities (ITs) where the muscle-bone interface forces are greatest [Breuls 2003; Drummond 1982; Ferguson-Pell 1980]. Ischial ulcers represent 24% of the total incidence of pressure ulcers [Brown 1990] and account for 59% of all recurring ulcers [Bates-Jensen, 2009]. Deep scar formation from a primary ulceration makes the tissue susceptible to future pressure ulcer development [Woolsey 1991], resulting in the high recurrence rate observed. The mismatched mechanical properties of scar tissue with the

surrounding muscle increases the pressure load at these interfaces, thus increasing the risk of induced damage.

1.2.2 Impact

Pressure ulcers are one of the most common secondary complications faced by individuals with SCI [Anson 1996; Walter 2002; Whiteneck 1992], and are a leading cause for re-hospitalization [Cardenas 2004; DeVivo 1998; Middleton 2004; Savic 2000; Johnson 1996]. In those with SCI, pressure ulcers are responsible for almost 7% of all hospital readmissions and account for 30% of bed-days, requiring an average length of stay of 10 weeks [Middleton 2004]. Of all the complications requiring hospitalization for people with SCI, pressure ulcers necessitate the longest hospital stays [Savic 2000].

Further disability, impaired mobility, and fatal complications are also associated with pressure ulcers [Richards 2004]. On average, 8 percent of individuals who require hospitalization for their pressure ulcers die of complications such as septicemia [Thomas 2001; Reuler 1981]. This is especially tragic in light of the fact that these individuals have survived a spinal cord injury only to pass away from a health concern considered by many as a preventable issue.

1.3 Classes of Pressure Ulcers

The National Pressure Ulcer Advisory Panel (NPUAP) in the United States defines pressure ulcers as a “localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure in combination with shear and/or friction”

[Black 2007]. The two main classifications of pressure ulcers are surface ulcers and deep tissue injury.

1.3.1 Surface Ulcers

Surface ulcers arise from skin damage which can result from inadequate or excessive moisture, poor nutrition, or friction due to improper transferring methods [Dinsdale 1974; Vidal 1991; Salcido 1995]. If not managed properly, surface ulcers can extend through the skin into underlying tissues. The NPUAP classifies the progression of surface ulcers into deeper tissues using a 4 stage system (Figure 1-1a), ranging from discoloration of intact skin (Stage 1) to full-thickness skin loss (Stage 2), tissue damage extending to the underlying fat (Stage 3), and ulceration that includes the muscle and bone (Stage 4) [Lyder 2003; Black 2007]. Fortunately, surface ulcers are usually detectable during their early stages of development, and there are a number of preventative methods in place to limit their progression.

1.3.2 Deep Tissue Injury

Deep tissue injury (DTI) refers to ulcers originating in subcutaneous tissue, resulting from damage arising from persistent pressure [Donnelly 2005]. This injury results from the compression of tissue between a bony prominence and an external hard surface [Guthrie 1973; Swarts 1988; Woolsey 1991; Salcido 1995; Berlowitz, 2007]. The compression causes local cell death, and can ultimately lead to widespread tissue death. Striated muscle has a lower threshold for damage due to pressure as compared to fat and skin [Daniel 1981; Salcido 1995; Nola 1980; Bliss 1993]. Therefore, when soft tissue

is compressed, skeletal muscle degenerates first. This leads to cell death that progresses through the tissue in an 'inside-out' manner (Figure 1-1b). Substantial tissue breakdown within the muscle, fascia and subcutaneous tissue occurs before signs of injury manifest at the skin surface [Salcido 1994; Paget 1873; Witkowski 1982]. Because, unlike skin, muscle is a non-regenerative tissue, devastating and non-reparable wounds result from DTI. The resulting damage necessitates dramatic interventions such as surgical reconstruction or amputation [Richards 2004]. The exact prevalence of DTI versus surface ulcers is currently unknown; however, Garber et al. [Garber 2003] recently indicated that 56% of all pressure ulcers are stage 3 and 4 ulcers (Figure 1-1a). Furthermore, Berlowitz et al. [Berlowitz 2007] proposed that DTI is responsible for all stage 3 and stage 4 ulcers. This indicates that DTI is not a rare occurrence, but a very important and pressing health issue to overcome.

1.4 Mechanisms of DTI

Numerous studies have been conducted to improve the understanding of the etiology of pressure ulcers and particularly, DTI. The most studied and commonly accepted mechanism of tissue death in DTI involves ischemia-reperfusion and its associated injurious pathways. Another pathway, studied to a lesser extent is that of the direct effect of mechanical deformation on soft tissue. Both pathways are discussed below.

1.4.1 Tissue Health and Responses to Distress

The health of the tissue depends on the viability of its constituent cells. Cells perform a wide range of tasks to maintain homeostasis and optimal functioning of the physiological systems in the body. These tasks require a constant supply of energy. In order to maintain production of sufficient energy, cells require a steady supply of oxygen and nutrients, as well as constant removal of metabolic wastes. Capillaries subjected to pressures greater than their perfusion pressure become occluded, halting this necessary exchange. Extended loading causing prolonged ischemia compromises the cell's ability to function properly [Kosiak 1958; Kosiak 1959; Kosiak 1961]. Restoring blood flow to this injured tissue, while necessary, can paradoxically further the tissue damage [Grace 1994; Gute 1998; Peirce 2000; Tsuji 2005]. In fact, cellular damage following ischemia-reperfusion injury is more severe than the damage incurred after ischemia alone, and is a primary factor in pressure ulcer development [Grace 1994]. The duration of cell survival under limited blood flow varies with cell type.

Due to its high energy demands, muscle tissue is extensively vascularized, and in turn is especially sensitive to interruptions in blood flow [Breuls 2003; Gefen 2005; Linder-Ganz 2006]. Muscle's sensitivity to ischemia renders it more susceptible to injury and necrosis than skin, which in comparison is less vascular and has a lower metabolic rate.

Normally, the cell maintains internal processes by creating, storing and utilizing energy in the form of adenosine triphosphate (ATP) (Figure 1-2). Under normoxic conditions, two pathways, glycolysis and oxidative phosphorylation, work consecutively

to oxidize glucose completely and generate ATP. Glycolysis creates 2 moles of ATP by metabolizing 1 mole of glucose to pyruvate. In the presence of oxygen, the pyruvate enters the citric acid cycle, and undergoes a series of enzyme-assisted conversions which generate electrons. The electrons are shunted to a respiratory chain within the inner membrane of the mitochondria. The respiratory complex transfers the electrons down a chain of enzyme complexes, releasing large amounts of energy, which is harnessed by the cell to create 34 moles of ATP. The presence of oxygen is a necessary condition for the post-glycolysis process of oxidative phosphorylation since molecular oxygen is the final electron acceptor within the respiratory complex.

1.4.2 Ischemia -reperfusion Injury

In the absence of oxygen, as would occur following ischemia caused by prolonged tissue compression, the cell is unable to undergo oxidative phosphorylation. Under these hypoxic conditions, adequate ATP can be generated for a short period of time, either by using creatine phosphate as an ATP substrate or through glycolysis (Figure 1-2). Creatine phosphate donates a high energy phosphate to adenosine diphosphate (ADP), converting it to ATP. During the first three hours of ischemia, enough ATP is produced by this pathway to ameliorate the effects of losing oxidative phosphorylation. Beyond this window, creatine phosphate stores are exhausted and only glycolysis is available for minimal ATP formation. The depletion of energy stores is accelerated as the rate of energy production by these pathways cannot meet the rate of consumption (Haljamäe 1975; Harris 1986]. After 4 hours of ischemia, there is a 96% drop in ATP levels in muscle cells [Tupling 2001].

With this drastic drop in ATP stores, many cell functions required for homeostasis are halted (Figure 1-3). Ion pumps that are dependent on energy to maintain necessary ion gradients across the cell membrane (such as the Na^+K^+-ATPase pumps) fail. K^+ freely diffuses out of the cell while Na^+, and Ca^{2+} accumulate inside the cell accompanied by water [Farber 1982], leading to cell swelling and eventual bursting of the membrane. Ca^{2+}, a potent secondary cell-signaling molecule, is normally sequestered in the mitochondria. Unexpected surges in Ca^{2+} activate Ca^{2+}-dependent proteases, nucleases, and phospholipids. This can lead to cellular dysfunction on a large scale and can also trigger apoptosis directly [Isozaki 2000].

The absence of blood flow alone has damaging effects. Without blood flow, glycolytic by-products such as lactic acid and the associated decrease in pH create cytotoxic conditions. In fact, lactic acid levels increase by 1000% over 4 hours of ischemia [Tupling 2001a; Tupling 2001b; Kabaroudis 2003]. This induces clumping of nuclear chromatin and inactivation of DNA and enzymes [Grace 1994; Tupling 2001a], thereby compromising cellular function.

Death of skeletal muscle is highly correlated to the level of ATP depletion which accordingly, is based on the period of ischemia. In fact, the relationship between the period of ischemia and extent of tissue necrosis approaches an exponential curve. Labbe et al. [Labbe 1987] found that 3, 4, and 5 hours of ischemia respectively resulted in 2, 30 and 90% of the skeletal muscle affected by necrosis. The depletion of substrates for alternate methods of ATP production described earlier explain why the damage

progresses from mild at 3 hours (2% necrosis) to extensive within 5 hours (90% necrosis).

The extent of damage is also dependent on the tissue's ability to restore ATP levels following ischemia. Skeletal muscle can quickly reestablish pre-ischemic levels of ATP following ischemic periods of less than one hour, allowing the cell to resume normal function. However, tissues subjected to ischemia for longer than 4 hours take much longer to regenerate their energy supply as ATP formation itself costs energy, and cellular ATP levels are exhausted after 4 hours of ischemia [Kabaroudis 2003].

The hypoxic conditions of ischemia also induce the formation of other deleterious molecules. Without oxygen, the cell's ability to convert ADP to ATP is substantially reduced. The increasing levels of ADP are broken down into hypoxanthine. Furthermore, the enzyme that normally breaks down hypoxanthine, xanthine dehydrogenase (XDH), undergoes an ischemia-induced conversion to xanthine oxidase (XO). Therefore, hypoxanthine accumulates during ischemia [Gute 1998; Kaminski 2002] and combines with oxygen upon reperfusion to form radical oxygen species (ROS). Longer periods of ischemia generate higher levels of ROS precursors, and upon reperfusion, overwhelm the cells oxygen free radical scavengers [McCord 1985; Meneshian 2002]. ROS such as superoxide and the hydroxyl radical cause oxidative damage to lipids, enzymes, proteins and DNA, and also trigger cell death directly [Li 2002]. Thus, while the restoration of blood flow is critical for cell and tissue survival, following prolonged periods of ischemia, it can further the injury [Reimer 1983; Grace 1994] (Figure 1-4). In fact, it is established that the reintroduction of oxygen after

ischemia is the primary cause of injury [Korthuis 1989], and makes ischemia-reperfusion injury more detrimental than ischemia alone [Parks 1986]. Oxygen's damaging role post ischemia is illustrated by the significant reduction in cell damage and skeletal muscle necrosis observed when using deoxygenated blood or reintroducing oxygen gradually into the reperfusate [Korthuis 1989]. Other studies in which free radical scavengers were added to deoxygenated blood resulted in further reductions in the levels of post-ischemic necrosis [Walker 1987].

Another significant factor resulting in cell and tissue death in reperfusion injury is damage to the endothelium, a single layer of cells lining all the vasculature in the body. Distressing stimuli including ischemia, shift the endothelium from a 'rest' condition to an 'activated' one [Vallet 2002], which produces a pro-inflammatory state. Leukocytes and platelets migrate to the distressed site, and release autolytic enzymes and ROS, damaging the microvasculature, increasing its permeability and causing edema [Braide 1984; Korthuis 1985; Weiss 1989]. This further adds to the structural and functional damage caused by the ROS [Labbe 1987; Grace 1994; Gute 1998; Appell 1999; Kabaroudis 2003].

One established phenomenon of the inflammatory response to ischemia-reperfusion injury is the 'no-reflow' effect [Menger 1992], in which even after reperfusion has been established, areas of tissue remain ischemic. Similarly to other mechanisms of injury within the reperfusion phase, the period and severity of ischemia determines the extent of the no-reflow induced damage [Hardy 1990]. A number of factors are interposed to create this phenomenon. During reperfusion, endothelial cell

swelling reduces the diameter of the capillary lumen. Activated leukocytes travel slowly through these capillaries due to their expression of various adhesion molecules. They accumulate easily and occlude the capillary, thus hindering reperfusion [Engler 1986; Schmid-Schönbein 1987]. Furthermore, the slow speed of the migrating leukocytes allows them a more intimate interaction with platelets thereby increasing the incidence of microvascular thrombosis [Cooper 2004].

Coagulation and thrombosis within the microvasculature can restrict delivery of nutrients and removal of waste products despite reperfusion [Hartsock 1989]. The improvement in tissue viability observed by administering blood thinners following ischemia [Hobson 1988; Belkin 1989] provides evidence for the role of coagulation and thrombosis in post-ischemic damage.

1.4.3 Mechanical Deformation

Two main stresses occur in the tissue of a seated individual. Compressive stress (or pressure) is generated perpendicularly to the sitting surface by the compression of tissue between a bony prominence and an external surface. If the angle at the hips is greater than 90°, shear stress may also be generated. In such a reclined position, an individual is prone to sliding down the surface, and tissue layers can incur damage as they slide over each other in opposite directions. Both stresses contribute to the formation of DTI [Kosiak 1961; Dinsdale 1974; Reswick 1976; Daniel 1981; Oomens 2003; Linder-Ganz 2007].

It is difficult to isolate the effects of mechanical damage due to pressure from the effects of ischemia in living systems, because pressure levels capable of inducing mechanical injury would also occlude the vasculature and cause ischemia. However, in vitro studies in single muscle cells [Peeters 2004; Peeters 2005] showed that when subjected to pressure, cells undergo deformation until a critical level of stress is reached. At this point, the cytoskeleton (scaffolding of the cell which provides structural support) uncouples from the cell, and the membrane ruptures causing cell death.

The amount of damage occurring in the muscle is dependent on two factors: the level of pressure applied, and the duration of this pressure [Kosiak 1961; Dinsdale 1974; Reswick 1976; Daniel 1981]. There is no conclusive evidence providing a threshold level of pressure or duration beyond which the onset and extent of injury can be accurately predicted. Furthermore, there is growing evidence that surface pressure measurements alone are incapable of predicting the risk of developing DTI [Bouten 2003; Oomens 2003; Agam 2007; Gefen 2007]. In theory and in practice, it is difficult to predict exactly how soft tissue as a whole responds to stresses like pressure and shear, as many factors affect the final stress profile observed. Soft tissue is comprised of heterogeneous, viscoelastic materials, and is capable of withstanding some degree of deformation without suffering irreversible damage. The constituent cells of each tissue layer possess differing mechanical structures, conferring each layer with unique mechanical properties. The health of the cell, its location and orientation with respect to other tissue and bone also affect the stress profiles observed. Prolonged periods of high stress induce changes in the muscle's mechanical properties, rendering the muscle stiffer. This stiffness increases the stress transmitted to adjacent, uninjured muscle [Linder-Ganz

2004; Gefen 2005]. Unfortunately, this results in a larger volume of muscle subjected to high levels of mechanical stress, further perpetuating DTI.

1.4.4 Interaction between Vascular and Mechanical Pathways of Damage

The independent contribution of ischemia-reperfusion and mechanical deformation to DTI is currently unknown. A recent animal study [Stekelenburg 2007] compared the effects of mechanical deformation against those of ischemia on the formation of DTI. Loading muscle for 2 hours (thus inducing mechanical damage and ischemia-reperfusion injury) created irreversible injury to the tissue, whereas 2 hours of ischemia alone resulted in minor damage that disappeared within an hour post load removal. Damaged tissue areas correlated to those experiencing the highest strains. It is likely that the initial onset of DTI is caused by the mechanical deformation of the tissue, and as time progresses the amount of damage is compounded with injury caused by the ischemia-reperfusion cascade.

1.4.5 Measurement Tools

Appropriate measurement tools are required to understand fully how and why the injury occurs, as the etiology of DTI is complex. Pressure and ischemia-reperfusion result in reduced levels of oxygen. This hypoxic environment plays a critical role in the progression of DTI. Accordingly, a brief discussion of the tools currently used to measure pressure and tissue oxygenation is warranted.

1.4.5.1 Pressure Measurement Tools:

Ideally, assessing the efficacy of an intervention to prevent DTI would measure the ability to reduce pressure at the bone-muscle interface, the place of origin of DTI. Unfortunately, there is currently no clinically viable method of measuring pressure at the deep bone muscle interface. Only invasive methods for deep pressure measurement exist, and are not suitable for human studies. I relied upon surface measurements of the IT pressure to evaluate the effectiveness of IES in reducing pressure at the bone-muscle interface. This is a commonly used alternative [Garber 1985; Henderson 1994; Burns 1999; Bogie 2003; Bogie 2006; Makhsous 2007; van Londen 2008], but the results do not provide a direct assessment of pressure at the deep bone-muscle interface.

1.4.5.2 Tissue Oxygenation Measurement Tools

Typically, changes in tissue oxygenation are measured using near infrared spectroscopy (NIRS), or transcutaneous oxygen tension (TcPO2). In context of pressure relieving methods for the prevention of pressure ulcers, tissue oxygenation is most often measured using TcPO2 [Coggrave 2003; Bogie 2003; Mawson 1993].

TcPO2 uses a sensor at the level of the skin to measure the free oxygen available to the epidermis. This is equated to be proportionate to the oxygen in tissue under the skin, and surrounding the sensor [Jarm 2003]. Clinically, these types of measurements are used to assess indirectly the severity of ischemia in peripheral vascular diseases [Slagsvold 1994; Bunt 1996].

While TcPO2 measurements can help provide an understanding of how various interventions affect tissue oxygenation, the measurements are confined to the skin microvasculature and do not allow direct analysis of muscle tissue oxygenation. Furthermore, because oxygen must diffuse to the skin surface to be measured by the sensor, important temporal effects may be lost due to diffusion delays [Ledermann 2006].

NIRS indirectly assesses relative changes in oxygenation by measuring the absorbance of specific wavelengths of light by biological chromophores such as hemoglobin. NIRS is a popular method for measuring oxygenation due to its non-invasive nature. However, the outcome variables rely on various assumptions and approximations [Wolf 2007], and the accuracy of the results can be affected by a variety of factors. The penetration, absorbance and reflectance of the light in the tissue are directly dependent on the placement of the source and receiving optodes. Because of the variation in body composition between individuals, NIRS measurements obtained with identical optode placement will likely be inconsistent since they would represent oxygen levels in varying tissue depths [Meyer 2004]. In fact, it has been suggested that NIRS is not an appropriate model for measuring oxygenation in tissues deeper than 2-3 cm from the surface [Perrey 2008], as subcutaneous tissue can reduce the measurement sensitivity of NIRS. Matsushita et al. demonstrated that a layer of fat notably decreases the sensitivity of the measurement [Matsushita 1998].

Tissue oxygenation can also be assessed using magnetic resonance imaging (MRI). Assessment of time-resolved blood oxygen level dependence (BOLD) effects has proven

to be an effective, indirect method for assessing changes in oxygen levels in and throughout skeletal muscle [Hennig 2000; Meyer 2001]. The technique measures changes in signal intensity (SI) in T2*-weighted images. T2* is the decay rate of signal in the transverse plane, and arises due to changes in both water volume and inhomogenities within the magnetic field. In the blood, the molecule hemoglobin (which carries oxygen) exists in two forms, oxy-hemoglobin and deoxy-hemoglobin. Relative increases in the deoxy-hemoglobin concentration results in decreases in SI [Ogawa 1990]. This results from the fact that in the deoxygenated form, hemoglobin has an unshielded iron molecule, which creates inhomogeneties in the MRI magnetic field. This speeds the decay of the signal and lowers the SI.

BOLD measurements have been verified and correlated with both NIRS and TcPO2, but offers advantages over both techniques. In particular, BOLD allows for real time evaluation of oxygenation changes in deep tissue [Noseworthy 2003].

The relationship between tissue oxygenation and T2* is given by the following equation:

$$1/T2^* = 1/T2(o) + k\,[O_2]$$

Where T2(o) represent baseline levels of signal intensity, $[O_2]$ is the concentration of oxygen, and k is a constant. The equation can be solved for k, giving:

$$K = [1/T2^* - 1/T2(o)] / [O_2]$$

Here, k provides the factor relating the values of T2* and the concentration of oxygen.

Collectively, while BOLD itself is not a direct measurement of tissue oxygenation, it provides one of the most comprehensive methods for assessing changes in deep tissue oxygenation.

The Oxylite system (Oxford Optronix, Oxford, UK) is capable of measuring tissue oxygen directly, but requires invasive procedures, and currently has not been approved for use in humans in North America. The system consists of a probe inserted into the muscle, from which light is emitted by a flourophore. This light is quenched in the presence of oxygen, with the quenching being directly proportional to the oxygen tension in the vicinity of the probe. Thus, direct measurements of oxygen can be made based differences in light reabsorbed by the system.

1.5 Physiology Unique to SCI

In addition to limited mobility and sensation, individuals with SCI face a number of pathophysiological changes after injury, which increase their susceptibility to the pathways that lead to the formation of DTI.

1.5.1 Muscle Atrophy

After SCI, muscle below the level of the lesion can no longer be voluntarily engaged. There is a concomitant wasting of these muscles [Demirel 1998], which can

cause up to a 70% reduction in muscle mass over a lifetime of SCI [Giangregorio 2005]. This reduction in the tissue's natural cushioning abilities [Guthrie 1973] results in higher levels of stress and strain for those with SCI relative to people with intact spinal cords, particularly over the bony prominences of the ITs [Linder-Ganz 2008]. Furthermore, bone loss in the hip region is observed in individuals with SCI [de Bruin 2000; Rittweger 2006]. These changes can induce notable alterations in the curvature of the IT, as well as the morphology of the tissue compressed between these bones and a seating surface [Linder-Ganz 2008].

1.5.2 Perfusion Issues

High pressures in tissue over the bony prominences hinder regional blood flow [Bogie 1995; Kloth 1988] and place the tissue at risk for developing a DTI. The loss of contractions also results in the loss of the 'muscle pump' action, which assists in keeping the muscle perfused by propelling blood through the capillaries to deeper tissues. The loss of this action furthers the level of muscle atrophy. Other factors such as vascular degeneration and reduced sympathetic neural activity below the level of the lesion [Mathias 1992; Mathias 1992; Maimoun 2006], make it difficult to maintain blood pressure. As a result, the ability to maintain perfusion in compressed tissue is decreased [Schubert 1991].

1.6 Pressure Ulcer Prevention Methods after SCI

The annual cost of treating pressure ulcers in people with SCI alone is about $1.2 to 1.3 billion. The vastness of this sum becomes truly appalling when one considers that

developing and executing preventative measures would cost about one tenth of this sum [Jones 2003; Byrne 1996; Bogie 2000]. Much research has been dedicated to developing methods for pressure ulcer prevention, and these have primarily focused on reducing pressure.

1.6.1 Repositioning

A commonly prescribed method for pressure ulcer prevention is frequent repositioning. The effectiveness of repositioning depends heavily on compliance by individuals with SCI, or the care providers. De Laat found that despite implementing a hospital guideline for pressure ulcer prevention stressing the importance of repositioning, the incidence of pressure ulcers did not decrease [De Laat 2006].

Surprisingly, there is no conclusive evidence proving the value of repositioning [Moore 2009; Krause 2004]. Regardless, repositioning is believed to be a key element in prevention. While there is currently no known optimal patient repositioning schedule [Whitney 2006], wheelchair users are encouraged to do a variety of activities meant to relieve pressure, including wheelchair push-ups and side-to-side or back-and-forth leans [Merbitz 1985; Grip 1986; White 1989].

Wheelchair push-ups may be difficult to execute by people with SCI due to their limited movement capacity. Even for those with suitable upper body strength and stability, lifting oneself for any amount of time requires significant muscle strength [van Drongelen 2005], and may lead to other complications such as upper body injuries [van Londen 2008]. Data from 46 individuals with SCI participating in a seating clinic indicated that pressure reducing activities, such as leaning forward, leaning from side-

to-side or tilting back in a wheelchair to 65° or higher were more easily performed than lifting. Furthermore, these activities (wheelchair pushups, side-to-side leans, etc) need to be sustained for an average of 2 minutes to overcome the pressure-induced hypoxia (as measured by changes in TcPO2) [Coggrave 2003]. Back-and-forth and side-to-side leans activities do not completely alleviate pressure, as full offloading is not achieved. While the hypoxia may be relieved, the pressure over the ITs still exists, and may induce mechanical damage.

1.6.2 Pressure Reducing Surfaces

Other methods of prevention include a variety of static and dynamic pressure reducing cushions [Garber 1979; Marshall 1983; Garber 1985; Garber 1985; Ferguson-Pell 1986]. The compliant surface of static cushions allows regions of high pressure under bony prominences to sink into the surface, spreading the pressure to surrounding areas [Woolsey 1991], and reducing the pressure at the skin-surface interface. Cushions can substantially reduce the pressure at the seat-surface interface, thus increasing the time one can spend seated [Garber 1979; Marshall 1983; Garber 1985; Garber 1985; Ferguson-Pell 1986]. However, they do not completely eliminate pressure over the bony prominences, and thereby retain an element of risk for the development of DTI. To date, a review of the efficacy of wheelchair cushions in preventing pressure sores found only one randomized controlled trial, in which participants were followed for five months. The study found that the use of specialized cushions did not provide protection against pressure ulcers, as there were no significant differences between the incidence, location, severity or healing time in developing sores [Lim 1988].

Powered cushions achieve pressure redistribution by actively pumping fluid (water or air) so that the pressure within the cushion is continuously changing [Gray 1999]. Such dynamic cushions have significant drawbacks that limit their effectiveness. They may generate the sensation of "seasickness" in some people; and their dependency on compressors and power supplies limits the mobility of the users and their level of activity [Conine 1989].

Combining pressure relief mechanisms with other methods, such as care giver education also fails to produce notable results. Exhaustive methods for increasing quality of care in clinical settings by increasing education for clinicians and funding for interventions like repositioning and pressure reducing mattresses show minimal [De Laat 2006] or no change in pressure ulcer prevalence [Gunningberg 2008].

Taken together, no intervention has significantly reduced the incidence of pressure ulcers to date [Conine 1989; Garber 1991; Garber 1982; Krause 2004; Raghavan 2003; Salzberg 1996; Seymour 1985; Thomas 2003]. This may be due to an incomplete understanding of the etiology of pressure ulcers (especially DTI), inadequate methods of prevention, or both. In current practice, the risk of developing pressure ulcers is determined by measuring the pressure at skin-surface interface. However, research indicates that pressure levels are higher in deep tissues surrounding bony prominences than at the surface [Bouten 2003; Oomens 2003; Agam 2007; Gefen 2007]. Thus, interventions geared at reducing pressure at the surface may not be effective in preventing DTI, which begins in the deep tissue. Furthermore, there is currently no

clinically viable method to detect DTI, which makes the need for an effective prevention tool paramount.

1.6.3 Electrical Stimulation

The ability of electrical stimulation to enhance muscle strength and retard muscle atrophy in people with SCI [Baker 1983; Douglas 1991], makes it an attractive candidate for the prevention of DTI. Unlike prevention methods such as cushions to reduce pressure, electrical stimulation can directly address the pathological issues leading to pressure ulcers in the tissue.

One method of using electrical stimulation for the prevention of pressure ulcers is to induce lifting to relieve pressure. Ferguson et al. demonstrated that in seated individuals, stimulating the quadriceps (while restraining the legs), knee moments could be generated. These moments induced lifting, and reduced the pressure measured under the buttocks [Ferguson 1992]. Similar results were demonstrated when stimulating the hamstrings. The induced hip extension was sufficient to unload the buttocks, and significantly reduce pressure over the ITs [Kaplan 2007]. However, this method has significant concerns. In particular, exposure of the fragile bones in individuals with SCI to large torques could result in bone fractures or breakages.

Another method of electrical stimulation use for pressure ulcer prevention focuses on increasing the viability of the tissue most susceptible to DTI. Bogie et al. used implanted electrodes in the gluteal muscles to administer stimulation to people with SCI [Bogie 2003]. Following an 8-week stimulation paradigm, increased muscle bulk was

noted with a concomitant, significant decrease in interface pressure in the ischial region compared to pre-conditioning levels. Furthermore, there was a trend towards increased transcutaneous partial pressure of oxygen (TcPO2) in unloaded gluteal tissue. The major drawback to this method is that the beneficial effects of electrical stimulation are not immediate; several weeks are required to build up the muscle bulk. Furthermore, building up muscle bulk alone provides static relief, which improves cushioning and lengthens the time one can sit comfortably [Rischbieth 1998; Bogie 2000; Bogie 2003; Liu 2006; Liu 2006]; however, it does not dynamically reduce or redistribute the pressure.

Other forms of electrical stimulation have also shown beneficial effects. Mawson et al. administered high voltage pulsed galvanic stimulation (HVPGS) for 30 minutes to 29 subjects lying supine [Mawson 1993]. The TcPO2 levels at the end of 30 minutes of HVPGS were 35 % higher than baseline.

Levine et al. demonstrated in able-bodied individuals that surface stimulation of the gluteal muscles generates changes in muscle shape [Levine 1990]. Each electrically induced contraction redistributed pressure at the seating interface [Levine 1989] and reduced pressure under the ITs. Similar results have been demonstrated in individuals with SCI [Liu 2006; van Londen 2008].

Taken collectively, these results indicate that electrical stimulation could be a potential method for the prevention of pressure ulcers. By improving tissue health and reducing pressure over the ITs, electrical stimulation directly confronts two major risk factors for DTI development. Conventionally, these benefits are elicited with stimulation administered in a 'constant' mode, where the 'ON' period is at least as long as the 'OFF' period. For example, stimulation may be provided for 8 seconds on, 4 seconds off [Bogie 2006]. Due to their atrophied, fatigue prone muscles, people with SCI would need a conditioning period before the electrical stimulation could increase the muscle bulk or improve tissue viability enough to have beneficial effects [Bogie 2006; Liu 2006].

A recent study has shown that gluteal contractions as short as 0.5 seconds can generate significant reductions in surface pressure over the ITs in people with SCI [van Londen 2008]. This paradigm utilized a duty cycle of 0.5 seconds 'ON', 15 seconds 'OFF' over the course of 31 minutes. These results are very encouraging, as they demonstrate that electrical stimulation can induce instantaneous reductions in pressure, allowing electrical stimulation to be used as an immediate tool for the prevention of pressure ulcers. However, this duty cycle may not be suitable for prolonged use in individuals with SCI, as typically after SCI, a 'slow-to-fast' transformation in muscle fibre phenotype [Burnham 1997; Talmadge 2002] occurs, rendering the muscle more prone to fatigue [Gallo 2004].

1.6.4 Intermittent Electrical Stimulation

The Mushahwar lab has been investigating a novel use of electrical stimulation for prophylaxis against DTI. The protocol employed, intermittent electrical stimulation (IES), is different from traditional philosophies of administering electrical stimulation. Rather than a constant administration of stimulation, periodic bouts of stimulation are utilized to induce postural adjustments. For example, a 10 second bout of stimulation, every 10 minutes (Figure 1-5a) is administered to mimic the movements able-bodied individuals subconsciously perform in response to discomfort while sitting.

IES is therefore also unique in its mechanism of action, as it is not dependent upon an increase in muscle mass for reduction in pressure. Instead, each IES-induced contraction provides periodic, dynamic pressure relief and redistribution, allowing for the technique to be used for long periods of time each day without risking muscle fatigue.

Previously, the effectiveness of IES in preventing pressure ulcer formation was tested in rats. The extent of DTI in muscles loaded for 2 hours was evaluated in the presence and absence of IES. The volume of DTI was significantly reduced in the muscles of the animals receiving IES relative to the untreated controls. In able-bodied human volunteers, IES periodically redistributed pressure and increased tissue oxygenation levels [Solis 2007; Solis 2008]. These results provided direct evidence for the potential capacity of IES to prevent pressure ulcers [Solis 2007]. Recently, a

preliminary study in able-bodied individuals extended the beneficial effects of IES [Solis 2008]. The study demonstrated that IES administered to the gluteal muscles induced significant reductions in pressure around the ITs, as well as significant increases in oxygen in the gluteus maximus muscles.

1.7 Overview of Masters Work

The focus of my thesis work was to investigate whether similar findings would occur in individuals with SCI. The main goal was to test two different IES paradigms and examine the changes induced by each in superficial pressure and tissue oxygenation.

1.7.1 Paradigms

The two paradigms consisted of either a continuous or bursting 'ON' period of IES. Within the continuous paradigm, two durations of 'ON' periods were tested, 7 and 13 seconds (Figure 1-5b). These durations were chosen because a 10 second 'ON' paradigm had demonstrated robust results in able-bodied individuals. I wanted to identify if a minimal stimulation duration is necessary to obtain the beneficial effects of each IES-induced contraction. Identifying this duration is of particular importance in people with SCI, as their atrophied muscles are prone to fatigue if over stimulated. The bursting paradigm consisted of three consecutive 3 second contractions, separated by 2 second breaks (Figure 1-5c).

1.7.2 Hypotheses

Based on preliminary results in able-bodied individuals, I hypothesized that IES would decrease superficial pressure around the ITs in seated individuals with SCI, and increase tissue oxygenation in the gluteus maximus muscles. In comparison to able-bodied individuals, the magnitude of IES-induced changes would be smaller given the atrophied state of muscles in people with SCI.

In other words:

H1: IES will significantly reduce the surface pressure from baseline levels in the tissue surrounding the ischial tuberosities in seated individuals with SCI.

H2: IES will significantly increase tissue oxygenation from baseline levels in the gluteus maximus muscle in seated individuals with SCI.

The null hypothesis is that IES will not decrease surface pressure or increase tissue oxygenation in seated individuals with SCI.

I further hypothesized these the increases in tissue oxygenation would occur in two ways; either due to an influx of blood flow via reactive hyperemia (continuous paradigm), or through the 'muscle pump' action (bursting paradigm). Furthermore, because the peak and duration of reactive hyperemia is inversely proportional to the degree of ischemia during muscle contractions [Humphreys 1963; Forrest 1989], I expected that the 13-second duration of stimulation would produce greater increases in tissue oxygenation than the 7-second duration in the continuous paradigm.

Chapter 2 of this thesis describes the experiments I conducted to address my hypotheses, the results I obtained and their significance.

Chapter 3 provides a general conclusion of my work, along with future directions for implementing IES as a clinical tool for the prevention of DTI.

1.8 Figures and Tables

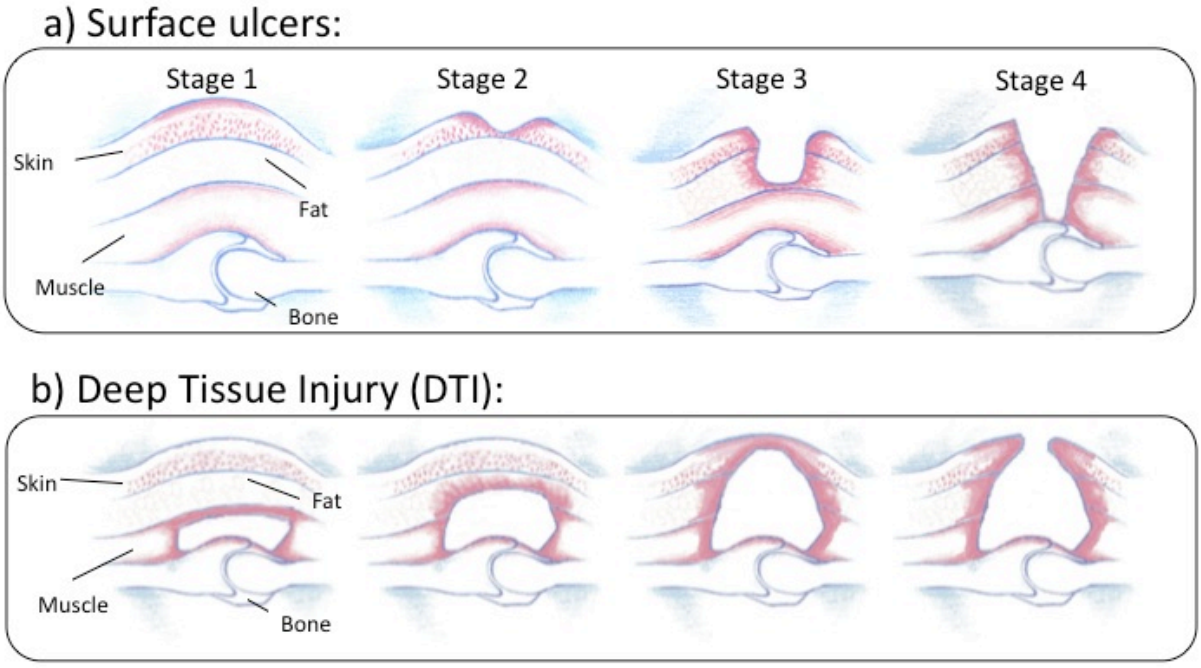

Figure 1-1: Classes of Pressure Ulcers

There are two categories of pressure ulcers: surface ulcers (a) begin at the level of the skin, and can progress inwards through 4 stages, and deep tissue injury (b) which begins at the bone-muscle interfaces, and progresses outwards.

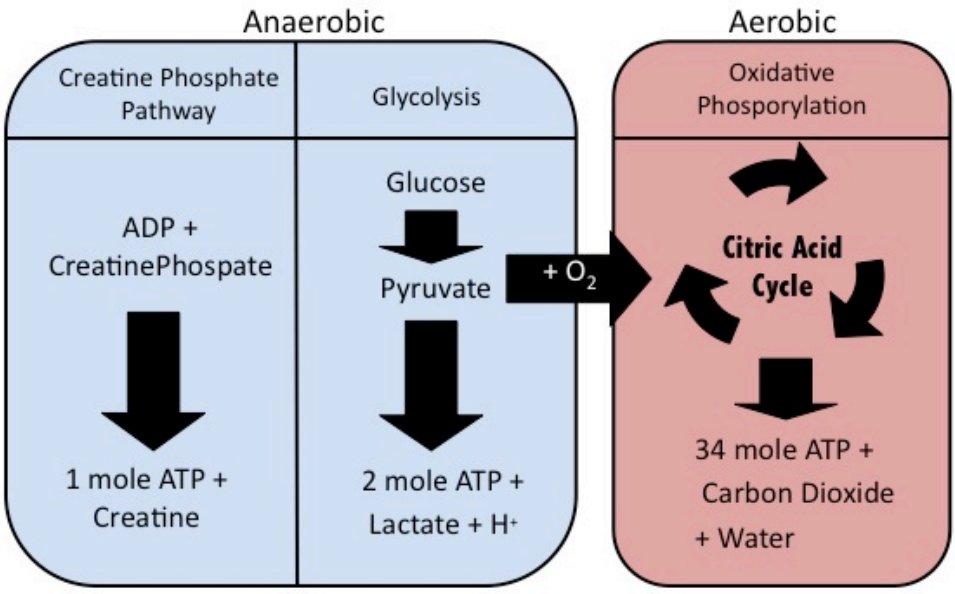

Figure 1-2: Energy Pathways of the cell

In the absence of oxygen, energy (ATP) is created via anerobic respiration, via two main pathways, glycolysis and the creatine phosphate pathway. In the presence of oxygen, energy is produced via aerobic respiration, a substantially more robust method of energy production.

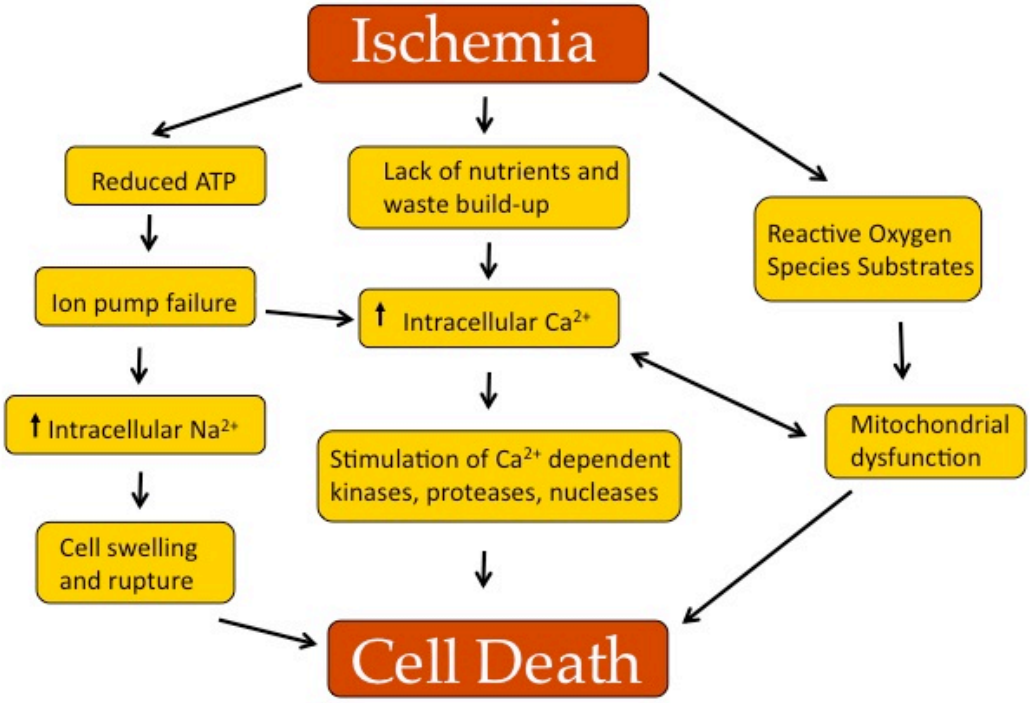

Figure 1-3: Pathological pathways in ischemia leading to DTI

When tissue is compressed , the vasculature can become occluded, thereby restricting nutrient delivery and waste removal. A number of detrimental events follow this restriction culminating in cell death, and resulting in DTI.

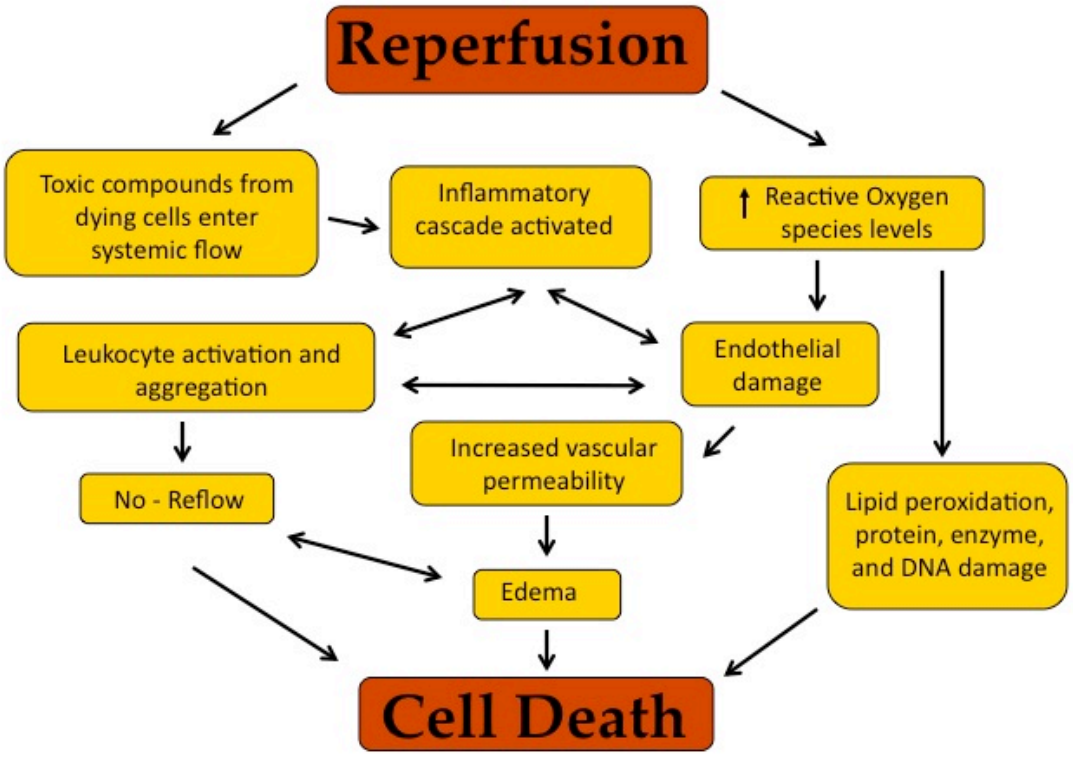

Figure 1-4: Pathological pathways in reperfusion leading to DTI

While the reintroduction of blood flow is paramount for the survival of tissue following ischemia, reperfusion (particularly the reintroduction of oxygen), can instigate a set of damaging events, also leading to cell death.

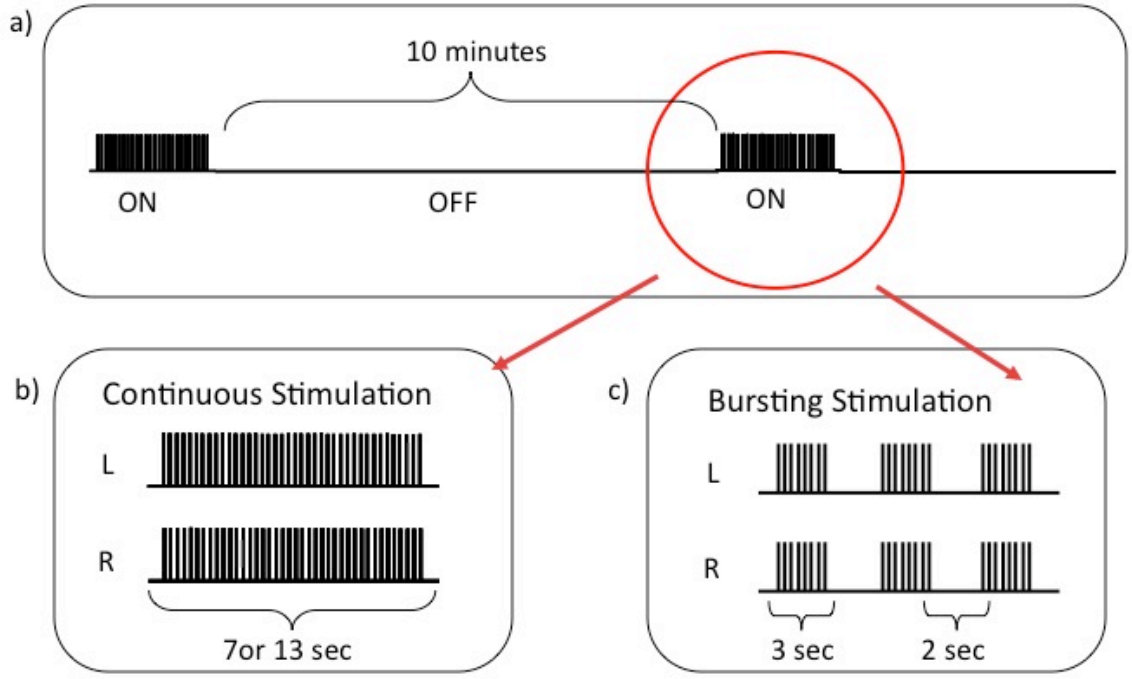

Figure 1-5: Intermittent Electrical Stimulation (IES)

IES consists of a stimulation 'ON' period of 7-13 seconds, and an 'OFF' period of 10 minutes (a). Two paradigms were tested, a Continuous stimulation (b) and Bursting stimulation (c). Within the continuous stimulation paradigm, two 'ON' durations were investigated, 7 and 13 seconds

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Chapter 2: Investigation of the Effects of Intermittent Electrical Stimulation on Surface Pressure and Deep Tissue Oxygenation in Individuals with Spinal Cord Injury

2.1 Introduction

Deep tissue injury (DTI) is a severe form of pressure ulcers that poses significant health risks for people with spinal cord injury (SCI). DTI develops in soft tissue compressed between a bony prominence and an exterior surface, such as the gluteus muscles. Following sustained loading, the compressed tissue breaks down due to pathological pathways arising from mechanical deformation, and ischemia and reperfusion injury.

Pressure ulcers are one of the most common secondary complications faced by individuals with SCI [Anson 1996; Johnson 1998; Klotz 2002; McKinley 1999; Noreau 2000; Walter 2002; Whiteneck 1992]. They are a leading cause for re-hospitalization [Cardenas 2004; DeVivo 1998; Middleton 2004; Savic 2000; Johnson 1996] and necessitate the longest stays [Savic 2000], requiring on average 10 weeks of hospitalization [Middleton 2004]. Each pressure ulcer costs $15,000 to $73,000 to heal [Rischbieth 1998; Grip 1986]. The estimated annual cost of treating pressure ulcers that develop during a hospital stay is $2.2 to $3.6 billion [Zanca 2003] in North America. Recently, the total cost of treating pressure ulcers in Canada was determined to be upwards of $3.5 billion [Ferguson-Pell 2008, personal communication]. Beyond the

financial consequences, pressure ulcers are associated with further disability, impaired mobility, and fatal complications [Richards 2004].

Much research has been dedicated to the field of pressure ulcer prevention. Methods such as weight shifting and pressure reduction surfaces including cushions and mattresses have been developed. Despite this, the incidence of pressure ulcers has not been effectively reduced [Conine 1989; Garber 1991; Garber 1982; Krause 2004; Raghavan 2003; Salzberg 1996; Seymour 1985; Thomas 2003].

The use of electrical stimulation for the prevention of pressure ulcers has demonstrated improved interface pressure distribution, reductions in muscle atrophy, as well as improvements in circulation, and transcutaneous oxygen [Bogie 2000; Bogie 2003; Bogie 2006; Baker 1983; Douglas 1991, Mawson 1993, Liu 2006; Levine 1989]. Traditionally, electrical stimulation has been used as a preventative method in two capacities. One method uses stimulation to induce lifting to relieve pressure. Stimulating the quadriceps while restraining the legs in seated individuals induced lifting of the hips and reduced the pressure measured under the buttocks [Ferguson 1992]. Similar results were demonstrated when stimulating the hamstrings [Kaplan 2007]. However, these methods have significant concerns such as the potential exposure of fragile bones in individuals with SCI to large torques that could result in bone fractures or breakages.

Another method focuses on improving tissue health and reducing pressure over the ischial tuberosities (ITs) by increasing muscle bulk. Typically, stimulation is

administered in a 'constant' mode, where the 'ON' period is at least as long as the 'OFF' period. For example, gluteal stimulation may be provided for 8 seconds on, 4 seconds off [Bogie 2006]. The resulting increases in muscle mass improve the cushioning capacity of the tissue and increase the length of time one can sit comfortably [Rischbieth 1998; Bogie 2000; Bogie 2003; Liu 2006; Liu 2006]. However, it does not dynamically reduce and redistribute the pressure, and requires a relatively long conditioning period before the increase in muscle bulk or improved tissue viability have beneficial effects [Bogie 2006; Liu 2006].

A recent study has shown that gluteal contractions as short as 0.5 seconds can generate significant reductions in surface pressure over the ITs in people with SCI [van Londen 2008]. This paradigm utilized a duty cycle of 0.5 seconds 'ON', 15 seconds 'OFF' over the course of 31 minutes. These results demonstrate that electrical stimulation can induce instantaneous reductions in pressure, allowing electrical stimulation to be used as an immediate tool for the prevention of pressure ulcers. However, the duty cycle employed would likely induce fatigue rapidly in people with SCI. To harness the immediate effects of electrical stimulation and beneficial long-term effects of improved tissue viability, a duty cycle is needed that can be used throughout the entire day of sitting without inducing fatigue.

Previous work in our lab has demonstrated that a novel method of electrical stimulation, referred to as intermittent electrical stimulation (IES), can prevent DTI [Solis 2007]. IES differs from traditional philosophies of administering electrical stimulation mainly in that the 'OFF' period in the duty cycle is on the order of minutes,

as opposed to seconds (Figure 2-1a). Short bouts of stimulation (for example, 10 seconds) are utilized to induce postural adjustments, mimicking the frequent adjustments able-bodied individuals make to alleviate discomfort due to prolong sitting. In between the bouts of stimulation, there are prolonged periods of rest (for example, 10 minutes), allowing for the technique to be used for long periods of time without risking muscle fatiguing. IES is not dependent upon an increase in muscle mass for the reduction in pressure, but rather, each IES-induced contraction provides periodic, dynamic pressure relief and redistribution.

Recently, a preliminary study in able-bodied individuals extended the beneficial effects of IES [Solis 2008]. The study demonstrated that IES administered to the gluteal muscles induces significant reductions in pressure around the ITs, as well as significant increases in oxygen in the gluteus maximus muscles.

The main goal of the current study was to investigate IES-induced changes in superficial pressure and deep tissue oxygenation in individuals with SCI. Two paradigms of stimulation during the 'ON' period of IES were tested: continuous and bursting. Both paradigms resulted in a consistent pattern of significant pressure reduction localized over the ITs. Furthermore, significant, sustained increases (up to 10 minutes) in tissue oxygenation were observed upon administration of each 'ON' period of IES with both paradigms. The increases in tissue oxygenation were verified in an animal model by using a direct, invasive method of measuring tissue oxygenation.

2.2 Methods

2.2.1 Effects of IES in individuals with SCI

2.2.1.1 Overview

All experimental protocols were approved by the Human Ethics Committee at the University of Alberta. Participants provided informed consent after the purposes and procedures of the experiments were explained to them. Each volunteer participated in two sessions, one assessing the effects of a specific IES paradigm on surface pressure while seated, and another assessing the effects of the same IES paradigm on tissue oxygenation in the gluteus maximus muscles.

2.2.2 Participants

Data were acquired from 17 individuals with SCI. Participant characteristics including age, gender, level of injury, extent of impairment (complete or incomplete) and year of injury are summarized in Table 1-1. Exclusion criteria included: existing pressure ulcers in the hip region, denervated gluteus maximus muscles, inability to withstand experimental levels of electrical stimulation, and contraindications for use of electrical stimulation or magnetic resonance imaging, such as magnetic metal implants, or implanted drug infusion devices.

2.2.3 Electrical Stimulation

At the beginning of each session, a research physiotherapist located the motor points for the gluteus maximus muscles bilaterally using a custom, sliding electrode. A 2-channel stimulator (BioMedical Life Systems Inc., Vista, CA, USA) was used to

administer current, biphasic, cathodic-first, charge balanced pulses, 200 μs in duration with a 40-Hz frequency to the gluteus maximus muscles for all volunteers. The threshold amplitude required to elicit a contraction, and the amplitude producing maximal contraction, were determined. Pairs of non-magnetic, 50X100mm surface electrodes (PureCare Inc., Sherwood Park, AB, Canada) were placed bilaterally on the gluteus maximus muscles, the cathode over the motor point for stimulation, and the anode more rostrally to serve as the return. The minimal stimulus amplitude producing maximal contraction was used. This always ranged between 40 and 120 mA.

2.2.4 Intermittent Electrical Stimulation Paradigms

Two stimulation paradigms of IES were investigated:

1. Continuous stimulation: This protocol consisted of simultaneous, bilateral, gluteal stimulation for either 7 or 13 seconds, followed by a 10 minute rest period (Figure 2-1b). These particular durations were chosen because a 10 second 'ON' paradigm had already been investigated in able-bodied individuals [Solis 2008]. Identifying the duration of the 'ON' period of IES is of particular importance in people with SCI, as their atrophied muscles are prone to fatigue if over stimulated.

2. Bursting stimulation: This protocol consisted of three simultaneous, bilateral bursts of stimulation to the gluteus muscles; each 3 seconds long with a brief 2 second rest period between each burst, again followed by a 10 minute rest period (Figure 2-1c).

These two paradigms were chosen to investigate the mechanism by which IES generates increases in oxygenation. Specifically, the continuous paradigm could increase

oxygenation through an influx of blood flow via reactive hyperemia. The bursting paradigm could increase oxygenation through the 'muscle pump' action.

Nine (9) individuals were assigned to the continuous stimulation group, and eight (8) were assigned to the bursting stimulation group. Participants in the continuous stimulation group underwent both the 7 and 13 second protocols to identify if a minimal stimulation duration is necessary to obtain the beneficial effects of IES-induced contractions.

2.2.5 Surface Pressure Measurements

Ideally, assessing the efficacy of an intervention to prevent DTI would measure the reduction in pressure at the bone-muscle interface. Currently, only invasive methods for deep pressure measurement exist, which makes them unsuitable for human experiments. Therefore, surface measurements of pressure were used to assess changes in pressure around the ITs during IES [Garber 1985; Henderson 1994; Burns 1999; Bogie 2003; Bogie 2006; Makhsous 2007; van Londen 2008].

The effect of each IES protocol on superficial pressure was tested by seating the volunteers on a Jay 2® composite gel/foam wheelchair cushion (Sunrise Medical, Longmont, CO). Pressure measurements were obtained using a pressure sensing mattress (XSENSOR, Calgary, AB, Canada) placed over the wheelchair cushion. The mattress was composed of a 36x36 array of 1cm^2 pressure sensors. Pressure profile maps were obtained from periods of sitting at rest, and while using the IES paradigms. Each paradigm was replicated 2-4 times depending on the participant's comfort level

during the experiment.

Each replicate consisted of the acquisition of a 5 sec period of baseline, the 'ON' period of the IES protocol, and 5 more seconds of rest. Between trials, 10 minutes of rest were allowed to keep in accordance with the pattern of IES. Pressure recordings were sampled at a rate of 10 frames per second. Measurements were calibrated between 0-200mmHg and 0-300mmHg depending on each participant's body weight, and the levels of pressure in the sensors under the areas containing the ITs, the areas of highest interface pressure for the volunteers. This ensured that none of the sensor readings was saturated, and that changes in pressure were detectable from baseline. All pressure measurements were imported into Matlab (Mathworks, Cambridge, MA, USA) for analysis.

For each IES trial in a given volunteer, pressure measurements were quantified from each sensor before and during the 'ON' period of IES. The values during the 'ON' period were compared to the baseline measurements for each sensor. The changes in pressure for each trial within the same paradigm were then averaged per volunteer. This provided a paradigm specific, sensor-by-sensor spatial image of where the significant pressure changes (increases and decreases) were for each individual. Finally, the pressure changes localized around the ITs were calculated as a percent change from baseline values. These values were averaged across all participants, and grouped by paradigm (continuous and bursting), allowing for the ratio of pressure change due to each paradigm to be determined.

A five-question survey was administered to individuals who retained some sensation to document any potential discomfort resulting from IES. Participants were asked to describe their level of discomfort before, during and after the 'ON' period of IES on a scale of 1-10 (1 being no discomfort, 10 being unbearable discomfort) for each trial. Participants were also asked to describe their perception of the IES as a preventative method of DTI, and any comments they had regarding it.

2.2.6 Oxygenation Measurements

To obtain an assessment of oxygenation within the gluteus maximus muscles, T2*-weighted magnetic resonance imaging (MRI) was used. T2*-weighted images are sensitive to changes in the levels of oxy-hemoglobin and deoxy-hemoglobin in tissue. These differences manifest as changes in signal intensity (SI). An increase in tissue oxygenation due to an IES-induced contraction is reflected by an increase in the SI of the muscle after the stimulation. These changes in SI can be quantified, and an indirect measurement of deep tissue oxygenation can be obtained [Hennig 2000; Meyer 2001].

All trials took place at the Peter S. Allen MR Research Centre at the University of Alberta, using a 1.5 T whole-body scanner (Sonata, Siemens Medical Solutions, Erlangen, Germany). Due to the limited space inside the magnet, the participants could not be scanned in a seated position. Therefore, to simulate the pressures experienced by the gluteal muscles during sitting, a custom-built, MRI compatible chair-like apparatus was used (Figure 2-2). The apparatus allowed variable levels of loading to be applied against the ITs and compress the gluteus muscles. Using this apparatus, the pressure profile of each volunteer in the magnet was matched to their seated pressure profile to

verify that any changes in tissue oxygenation seen due to IES in the magnet would also occur when seated in a wheelchair.

Each T2*-weighted imaging trial consisted of a 30 second baseline period, the 'ON' period of IES, and a post-stimulation period of 9 minutes and 20 seconds. The total length of each trial was 10 minutes. A spine array coil (2 elements), combined with a 2-element CP Body Array Flex coil (Sonata, Siemens Medical Solutions, Erlangen, Germany) were used for imaging the pelvic region. T2*-weighted images were acquired with the following parameters: echo time = 37 ms, repetition time = 1330 ms, number of slices = 15, slice thickness = 6 mm, slice separation = 12 mm, field of view = 400 X 275 mm, readout matrix = 88 X 128 pixels, in-plane resolution = 3.1 X 3.1 mm. The 15 slices provided coverage of the pelvic and upper thigh region. Over the time course of each trial (10 minutes), 450 sets of these slices were acquired. For analysis, the SI in the gluteus muscles at baseline was compared to the SI following the IES-induced contraction.

For each trial during the imaging session, the survey used during the pressure mapping session was re-administered to document any potential discomfort resulting from IES.

2.2.6.1 Temporal Changes in Oxygen due to IES

For each paradigm, 2-4 trials were obtained and averaged per person. The data were analyzed using a custom-written Matlab program (Mathworks, Natick, Massachusetts, U.S.A.). Of the 15 slices obtained, 8 containing the entirety of the gluteus

maximus muscle were used for analysis (Figure 2-3). A region of interest (ROI) was selected around the right and left gluteus maximus muscles in each slice, and the SI was determined (Figure 2-4). The average pre-contraction SI values were calculated over 30 seconds (baseline) and subsequent values were normalized to this value. Due to the variation and noise inherent in T2*-weighted measurements, the data were averaged into 30 second bins, and the SI values were expressed as a percentage change relative to baseline.

2.2.6.2 Spatial changes in Oxygen due to IES

A slice-by-slice analysis was completed to determine whether there were spatial differences in oxygenation levels relative to the tissue most compressed by the ITs. The SI values in the ROI for each of the 8 slices were assessed pre- and post-IES, and collapsed over time to represent the overall change in oxygenation in each slice.

2.2.7 Statistical Analysis

The percent change in pressure in each paradigm during the 'ON' period of IES was compared to baseline values using paired *t* tests. The changes were also compared to each other using a one-way analysis of variance (ANOVA). The changes in oxygenation due to the 'ON' period of IES were compared to baseline values and each other, using a one-way ANOVA. Differences were considered to significant for $p \leq 0.05$. Tukey's Honestly Significant Difference post-hoc analysis was utilized when significant changes were observed. All results are presented as mean ± standard error.

2.2.8 Oxygenation Measurements in a Rat Model

2.2.8.1 Overview

To obtain a better understanding of the changes in tissue oxygenation due to IES and correlate them to T2*-weighted measurements obtained in human volunteers, an experiment was conducted in a rat model of IES. All experimental protocols were approved by he Animal Care and Welfare Committee at the University of Alberta.

The profile of oxygenation changes following IES was first assessed using T2*-weighted images to verify that the increases in SI observed in the human study were not an artifact of movement or experimental protocol. In a separate experiment, direct measurements of tissue oxygen were obtained to correlate with the T2* values. In an effort to further characterize the effects of IES-induced changes on oxygenation, a combination of various contraction strengths and loading levels were assessed.

2.2.8.2 Experimental Setup

In both experiments, IES was administered to the gastrocnemius muscle of one hind limb via a bipolar cuff electrode, implanted around the tibial nerve. The rat was anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital (54.7mg/mL) at a dose of 45mg/kg. Following induction, anesthesia was maintained through 0.1mL IP injections of the same dose, administered as needed throughout the experiment. Following the implantation and testing of the nerve cuff the skin was sutured closed. The rat was then placed in an MR-compatible, custom-built apparatus in a prone position (Figure 2-5). The rat's foot on the experimental hind limb was rotated medially at a 45 $^{\circ}$ angle, placed in a plaster boot, and secured with a Velcro strap at the

ankle. This pushed the gastrocnemius muscle into an accessible position, and ensured an isometric contraction during stimulation. An indenter (3mm radius) compressed the gastrocnemius muscle around the midline and approximately one-third the distance from the knee to ankle joint. The indenter served as the IT, and the tibia acted as the external seating surface, effectively compressing the soft tissue in a manner similar to that seen in the seated condition [Solis 2007]. The indenter was designed to support various loads ranging from 10% to 100% of total body weight.

*2.2.8.3 Comparison and Verification of T2**

To assess SI changes due to IES in T2*-weighted images, the rat (in the apparatus) was transferred to the MR Centre. The gastrocnemius muscle was loaded using the indenter. Using a similar protocol to that in the human study, MR images were obtained while utilizing IES to induce a maximal, 10 second contraction. Stimulation consisted of biphasic, cathodic-first, charge balanced pulses, 200 μs in duration, delivered to the nerve cuff using a stimulator (BioMedical Life Systems Inc, Vista, CA, USA).

A 3-cm birdcage finger coil (Siemens Medical, Canada) was used to image the rat's hind limb. T2*-weighted images were acquired with the following parameters: echo time = 25 ms, repetition time = 500 ms, number of slices = 5, slice thickness = 3 mm, slice separation = 3.6 mm, field of view = 55 X 19 mm, readout matrix = 44 X 128 pixels, in-plane resolution = 0.43 X 0.43 mm. Over the time course of each trial (15 minutes), 1200 sets of these slices were acquired. For analysis, the SI in the gastrocnemius muscle at baseline was compared to the SI following the IES-induced contraction.

2.2.8.4 Correlation of T2 to Direct Oxygen Measurements*

The second experiment assessed direct oxygen changes following the utilization of IES. The strength of contraction and level of loading on the muscle were manipulated over trials to observe how these variables affect the levels of tissue oxygenation induced by IES. Following the implantation of the nerve cuff and placement in the apparatus, a fibre optic probe consisting of an oxygen sensor (Oxylite, Oxford Optronix, Oxford, UK) was inserted into the gastrocnemius muscle. The probe was inserted using a 20 gauge intravenous catheter (Canada Medical Ltd. Surgical Supplies, AB) and positioned approximately 3mm from midline, running parallel to the long-axis of the muscle at a depth of 2mm, situated adjacent to the indenter. A baseline of 15 min of stable readings was obtained before the trials began. Thereafter, each trial consisted of a 30 second baseline before IES was administered. Data were sampled by the fibre optic probe every 5 seconds, before, during, and after stimulation. These measurements were captured at a sampling rate of 10Hz using a data acquisition interface (Power 1401, Cambridge Electronic Design, Cambridge, UK) with associated software (Signal version 2.13, Cambridge Electronic Design).

Four trials were conducted: trial 1 – maximal contraction with a load of 18% of body weight (BW) applied to the muscle; trial 2 – repeat of trial 1; trial 3 – moderate contraction with 18% BW; and trial 4 – moderate contraction at 28% BW. The strength of the contraction was determined by visual inspection. A maximal contraction was considered to be the largest contraction elicited with a stimulus level beyond which further increases in stimulation did not produce stronger contractions. A moderate

contraction was considered to be a contraction greater than that elicited by threshold stimulus levels, but notably reduced from maximal contraction. Contraction strength was modulated by using either bipolar or monopolar IES administration.

2.3 Results

2.3. Effects of IES in individuals with SCI

2.3.1.1 Surface Pressure Mapping

In each group (continuous and bursting stimulation) data from one participant could not be used. In the continuous group, one participant had widely fluctuating changes in pressure between trials (both massive increases and decreases) due to an error in the connectivity within the pressure sensing system, which was subsequently corrected. In the bursting group, the data from one participant was excluded from the analysis because the ITs could not be localized on the pressure mapping images due to increased levels of adipose tissue. Thus, for the surface pressure mapping analysis, the continuous and bursting groups had an n=8 and n=7, respectively.

Examples of the baseline pressure profiles obtained during rest in seated volunteers with SCI are showed in Figure 2-6a. The profiles obtained during the 'ON' period of IES (Figure 2-6b) showed a reduction in pressure over the IT region in comparison to baseline levels (Figure 2-6a). This reduction appeared to be accommodated by an increase in pressure in the tissue away from the ITs. Both IES protocols (continuous and bursting) produced a consistent pattern of significant pressure reductions over the ITs (one way ANOVA, $p < 0.05$) (Figure 2-6c).

A trial-by-trial comparison was conducted to determine whether there were changes in the magnitude in pressure reduction over time. These types of changes could occur due to muscle fatigue, creep effects in the pressure sensing mattress, or the gel cushion interacting with the contracting tissue, dampening the levels of pressure reduction due to IES over time. Comparing the reduction in pressure in each trial established that no such changes over time occurred (data not shown) to a maximum of 50 minutes (longest experimental time).

Figure 2-7 shows the average percent reductions in pressure over the ITs for both the left and right sides of the buttocks. There were notable differences in the level of pressure reduction with stimulation in the two sides; however, these differences did not reach significance. In the continuous paradigm, the 13 second protocol produced a 9.8 ± 5.9% reduction in pressure on the left side, and a 10.2 ± 5.6% reduction in pressure on the right side, while the 7 second protocol produced an average 10.3 ± 5.1% reduction in pressure on the left side, and a 22.3 ± 12.7% reduction in pressure on the right side. For the bursting paradigm, there was a 24 ± 10.3% reduction in pressure on the left side and a 30.9 ± 14.9% reduction in pressure on the right side. All reductions in pressure were significant ($p < 0.05$), except for 13 seconds, left side ($p = 0.07$), and 7 seconds, right side ($p = 0.06$), both of which approached significance. The range of pressure reduction was 2-100% across individuals and paradigms.

When compared against each other, there was no significant difference between the pressure reduction in the 7 second, 13 second, or bursting paradigms on the left or

right sides (one-way ANOVA, p=0.5, p=0.6, respectively).

2.3.1.2 Tissue Oxygenation

The T2*-weighted images from 5 individuals were not suitable for analysis. Reasons for exclusion included noise from implants in the area imaged, improper loading, and spasticity causing movement artifact and changes in SI not due to IES. Accordingly, for the tissue oxygenation analysis, each IES paradigm (continuous and bursting) included an n=6.

MRI images confirmed that both paradigms of IES produced a visible contraction and reconfiguration of muscle shape in all volunteers. Figure 2-8 shows a T2*-weighted, transverse image through the hip region of a single participant as an example of the changes in muscle shape induced by the 'ON' period of IES. The three consecutive slices in the top row show the muscle configuration at rest (Figure 2-8a) and the same three slices in the bottom row show the muscle configuration during IES -induced contraction (Figure 2-8b).

IES resulted in an increase in oxygenation in the gluteal muscles compared to baseline in both paradigms. In general, utilization of IES resulted in a step-wise increase in oxygenation levels over trials, particularly in individuals whose trials were run consecutively (i.e., without repositioning or interruptions). In effect, the gains from each trial built upon the previous trial, creating a shifting baseline (Figure 2-9). One observation of note, as shown in the figure, is that participant 2 (Figure 2-9) felt that she may have an episode of autonomic dysreflexia due to a full bladder after the second trial.

The disproportionately high SI observed in the third trial, likely reflects a systemic increase in oxygen due to activation of the sympathetic nervous system, as well as the increases due to IES. After emptying her bladder, the SI levels returned to a level in line with the rate of increase due to IES.

Both 'ON' durations in the continuous group (13 and 7 seconds) produced similar results: eliciting a large initial peak, which stabilized at a lower value over time. The bursting paradigm did not produce an initial peak; instead the 'ON' period of IES resulted in a large initial increase that was maintained for the entirety of the scan (Figure 2-10).

Immediately following IES, there was a significant elevation in SI relative to baseline (one-way ANOVA, $p < 0.05$) for both paradigms. The SI remained significantly elevated from baseline for the duration of each 10-minute scan (one-way ANOVA, $p < 0.05$) (Figure 2-11). The maximum increase observed for the 13 second, 7 second and bursting durations was respectively 3.4 ± 0.01%, 2.6 ± 0.01% and 2.0 ± 0.01%, while the average overall increase following stimulation was respectively 2.1 ± 0.13%, 1.4 ± 0.10% and 1.9 ± 0.11%.

The increases in oxygenation following the 7 second continuous and bursting paradigms were not significantly different from each other during the first 30 second data bin. Thereafter, the 7 second stimulation was significantly lower than the other two paradigms (but significantly elevated from baseline) for the remainder of the scan. The levels of increase in SI for the 13 second stimulation and the bursting stimulation

overlapped at various time points, particularly between minutes 5 and 8 of the scan. For these two paradigms, the increases in oxygenation were not significantly different from each other at time 3.5 min, 5.5 – 8.0 min, and 9.5 min (one-way ANOVA, $p > 0.05$). At all other time points, increases in oxygenation due to 13 second continuous IES paradigm were significantly higher than those induced by the bursting paradigm (one-way ANOVA, $p < 0.05$).

A slice-by-slice analysis to determine whether there were spatial differences in oxygenation levels relative to the tissue most compressed by the ITs was conducted. Surprisingly, no spatial patterns of tissue oxygenation were found (Figure 2-12).

2.3.2 Qualitative Assessment of IES

The results from the qualitative survey universally revealed that IES did not produce any sensations of discomfort. None of the volunteers complained of somatic pain or distress due to IES, and typically volunteers noted that IES induced a pleasant 'vibration,' felt viscerally. Furthermore, participants stated that IES alleviated their discomfort after prolonged sitting, and some suggested that the duration of IES be increased to enhance this effect. One volunteer indicated that his persistent neuropathic pain was reduced by IES more than other interventions he had attempted.

2.3.3 Oxygenation Measurements in a Rat Model

2.3.3.1 IES-induced Effects on SI

In a rat in which IES was delivered to the tibial nerve through an implanted nerve cuff, IES resulted in increases in SI in T2*-weighted images. SI values in the stimulated gastrocnemius muscle and in an adjacent, non-stimulated muscle were compared. Increases following the 'ON' duration of IES were observed in the stimulated muscle, but not the non-stimulated muscle (Figure 2-13).

The pattern of increases in SI was similar to that seen in the human investigations (Figure 2-13). This indicates that the rat model provided an appropriate approximation of IES-induced SI changes in the human study. Because the animal's position, movement, and sedation were rigorously controlled, this experiment confirmed that the increases in SI in the human experiments were not due to movement artifact induced by stimulation.

The maximal increase in SI observed after the 10 second 'ON' duration of IES in the rat was 14.88 %, while the average overall increase following stimulation was 6.7 ± 0.10%.

2.3.3.2 IES-induced Effects on Direct Oxygen Levels

Four trials were conducted to assess direct oxygen changes in a separate rat (Figure 2-13). Following Trial 1 (maximal contraction and 18% BW), there was an immediate 98% increase in the levels of oxygen in the tissue surrounding the probe. While this initial level decreased over time, the oxygenation levels remained above the

original baseline levels for the duration of the trial (15 minutes). Consequently, the baseline for Trial 2 (repeat of Trial 1 variables) was almost 2 fold higher than the baseline for Trial 1. All subsequent trials began and returned to this elevated baseline (Figure 2-14; Figure 2-15a). In Trial 2, an immediate 38% increase in tissue oxygenation was observed. The increases in oxygenation gradually returned to the new, elevated baseline levels by the end of the trial. Interestingly, the next trial, conducted with a moderate contraction strength, produced a 37 % increase in oxygenation relative to baseline, the same proportion of increase as the previous trial (Figure 2-15b). Again, the oxygen levels returned to baseline at the end of the 15-minute trial. The fourth and last trial was conducted with the same moderate contraction strength, but with increased loading (28% BW). This produced the smallest gains post stimulation (Figure 2-15b). This indicates that the level of the loading has a notable effect on the capacity of IES to induce increases in tissue oxygenation. Taken collectively, this preliminary study suggests that the 6-15% increase in SI observed during IES-induced contractions in compressed tissue is comparable to a 19 - 98% increase in muscle oxygenation.

2.4 Discussion

2.4.1 Overview

The overall goal of this study was to investigate the effects of IES on surface pressure and deep tissue oxygenation in individuals with SCI. The results demonstrate that IES in this population produces significant reductions in pressure over the ITs and significant, sustained increases in tissue oxygenation.

Notably, these effects occur under loaded conditions and in atrophied muscles that had not been conditioned prior to the experiment. The magnitude of the reductions in pressure did not diminish over time, nor did the increases in tissue oxygenation. Furthermore, the step-wise increases in oxygenation observed in participants whose trials were run consecutively indicate that a 1-4% increase in SI occurred each trial (Figures 2-9, 2-10). Accordingly, our presentation of the overall average increases in tissue oxygenation due to IES is a conservative estimate, because the normalization of SI values to baseline for each trial removed the sustained increases observed between trials.

The main goal of the rat study was to verify and correlate the changes in SI observed in the human study using direct measurements of oxygen. While conducted in a very limited sample (n=2), the findings are very revealing. The results verified that the increases in oxygenation observed in the human study are due to IES. The results also suggested that there are many factors that affect the levels of increase in tissue oxygenation, including history, contraction strength, duration between stimulation bouts and total load on the muscle.

Collectively, the results suggest that the IES protocol, which consists of short stimulation bouts and long rest periods, may be an effective method for preventing DTI in people who have fatigable muscles, such as individuals with SCI.

2.4.2 Comparisons with Previous work

A preliminary study in 5 able-bodied individuals seated on an office chair

demonstrated that IES induced significant reductions in pressure around the ITs [Solis 2008]. The results from the present study parallel the effects of IES observed in able-bodied individuals. This is compelling given the muscles in SCI are significantly atrophied compared to those in able-bodied individuals. Therefore, the utility of IES is independent of muscle bulk.

Other pressure reducing activities, such as wheelchair pushups (which need to be conducted for 2 minutes to alleviate hypoxia) or electrical stimulation to generate torque and induce lifting, can be difficult or dangerous for individuals in wheelchairs to undertake. IES uniquely allows for immediate, dynamic pressure reduction in a manner mimicking the constant repositioning and postural readjustments conducted by able-bodied individuals to relieve pressure. Such relief of pressure is important given that all of the pathological processes leading to DTI stem from pressure-based damage to deep tissue.

Whether or not the reduction in pressure observed over the ITs during IES would be sufficient to prevent DTI in people with SCI is currently unknown. This is so because the changes in pressure at the bone-muscle interface could not be measured. Surface pressure at the skin/seating interface was measured instead and currently, there is no established absolute value that surface pressure must be reduced to for the prevention of pressure ulcers [Brienza 2001; Eckrich 1991]. Thus, surface pressure measurements do not provide a complete description of the reductions of pressure at the deep bone-muscle interfaces, the site of DTI. Nonetheless, results from a previous study in rats utilizing IES in loaded tissue over two hours demonstrated that IES was effective in

preventing DTI during that time period [Solis 2007]. This strongly suggests that IES is capable of inducing adequate reductions in pressure at the bone-muscle interface.

The IES-induced increases in oxygenation observed in individuals with SCI were also similar to those observed in able-bodied individuals using IES [Solis 2008]. Following a 10 second 'ON' period of IES, the tissue oxygenation in able-bodied individuals rose to a maximum of 3.2% relative to baseline. In fact, it is interesting to note that the pattern of changes in tissue oxygenation seen for the 13, 10, and 7 second paradigm of the 'ON' period of IES is approximately linear (3.4, 3.2, and 2.6%, respectively) despite the fact that the changes in the 13 and 7 second paradigms were in individuals with severely atrophied muscles, and the results from the 10 second paradigm were obtained in able-bodied individuals [Solis 2008].

This study is the second part of an investigation [Solis 2007] which, to the best of our knowledge, is the first to measure muscle oxygenation following contractions in *compressed tissue*. The clinical relevance of these findings is particularly compelling, as the sustained increases are demonstrated in individuals with SCI who are at high risk of developing DTI. Various studies have shown increases in muscle oxygenation following contraction, but despite having similar contraction durations, the time course of the increases in SI observed differ substantially [Damon 2007a; Damon 2007b; Sanchez 2009; Meyer 2004]. The difference observed is likely due to the fact that the tissue is not compressed in those aforementioned studies.

2.4.3 Mechanism of Action of IES

We originally posited that the mechanism underlying the increases in tissue oxygenation obtained by IES was based on changes in blood flow following the induced contractions (i.e., reactive hyperemia or muscle pump action). However, increases in oxygenation due to blood flow occur on the order of seconds, and cannot explain the sustained elevations observed in our results. Other studies using similar paradigms (1 – 10 second contractions in unloaded muscle) have demonstrated transient increases in SI, lasting on the order of tens of seconds [Damon 2007a; Damon 2007b; Sanchez 2009; Meyer 2004]. The time course of the initial peak in SI observed following the 7 or 13 second contractions is similar to that observed in other studies [Brock 1998; Hennig 2000], and is attributed to a contraction-induced reactive hyperemia [Brillault-Salvat 1997; Duteil 2004; Carlier 2005]. In agreement with previous work [Humphreys 1963; Forrest 1989], the reactive hyperemia observed following the 13 second bout of stimulation was greater than that following the 7 second bout of stimulation. The 10 second bout of stimulation assessed in able-bodied individuals fell between these values [Solis 2008].

However, the mechanism for the sustained elevation in tissue oxygenation following the initial peak is currently not fully understood. One possible explanation is that in response to a stressful event, there is a reduction in the muscle's metabolic rate, thereby decreasing its oxygen and energy requirements [Jordan 2004]. This would cause a relative increase in the oxygenation levels in the microvasculature of the tissue, increasing the SI in T2*-weighted images, as observed in the current study.

An example of this protective response to mild stress is the phenomenon of ischemic preconditioning in which short periods of ischemia provide protection against prolonged, future ischemic events [Murry 1986; Saito 2004]. In an experimental porcine model, ischemic preconditioning reduced necrosis in skeletal muscle by 55% following an extended ischemic event [Mounsey 1992]. It is believed that ischemic preconditioning provides this protection by reducing cellular metabolism, reducing inflammation and ameliorating the no-reflow phenomenon [Jerome 1995; Akimitsu 1995; Akimitsu 1996].

This could partially be the mechanism of action for IES because each electrically induced contraction constitutes a transient ischemic event. The miniature ischemic events may drive the muscle towards a 'protective' state, reducing the metabolic requirements of the tissue. Once in a protective state, the muscle is able to survive under prolonged durations of ischemia due to compression, despite the lack of nutrients and energy. This would explain not only the sustained elevations in tissue oxygenation, but also the ability of IES to prevent the breakdown of loaded muscles in a previous animal model of DTI [Solis 2007].

Nitric oxide (NO) may be partly involved in the protective state induced by IES [Reid 1998; Radegran 2000, Jordan 2004]. NO has a wide range of effects in the body, and is produced during muscle contractions. It competes with oxygen for the active site of cytochrome c oxidase, which can inhibit mitochondrial respiration [Brown 2001; Reid 1998; Shen 1995; Moncada 2002], thereby limiting oxygen consumption (and requirements) by muscle cells. Blocking nitric oxide synthase leads to an immediate

reduction in muscle oxygenation following exercise, with a similar time course as the return of blood flow to basal levels [Jordan 2004].

2.5 Conclusion

In summary, this study demonstrated that IES effects significant pressure reductions over the ITs, and increases oxygenation in the stimulated muscle. The increases in oxygenation were verified by a preliminary study in rats.

These results indicate that IES may be an effective method for the prevention of DTI by directly ameliorating the pathogenic factors contributing to the development of pressure ulcers.

2.6 Figures and Tables

Volunteer	Age	Gender	Level of Injury	Type of Injury	Extent of impairment	Year of Injury	Paradigm
A	40	female	T4-T5	SCI	complete	1999	Continuous
B	32	male	C1-C2, T3	SCI	complete	2006	Continuous
C	30	male	C4	SCI	complete	2003	Continuous
D	33	female	C6-C7	SCI	complete	2007	Continuous
E	66	male	T4	encephalitis	complete	1997	Continuous
F	31	female	C6-C7	SCI	complete	2007	Continuous
G	32	male	C6-C7	SCI	complete	2001	Continuous
H	22	male	C5-C6	SCI	complete	2005	Continuous
I	28	female	C5	SCI	complete	2007	Continuous
J	31	male	C6-C7	SCI	complete	1999	Bursting
K	45	male	T4	SCI	complete	1989	Bursting
L	57	male	C7-C8	SCI	complete	1978	Bursting
M	42	male	C3-C4	SCI	complete	1987	Bursting
N	35	female	C5	SCI	complete	2003	Bursting
O	25	female	T5	SCI	complete	2000	Bursting
P	31	female	C6-C7	SCI	complete	2007	Bursting
Q	53	male	C7-T1	non traumatic SCI	Incomplete	2003	Bursting

Table 2-1: Participant Characteristics

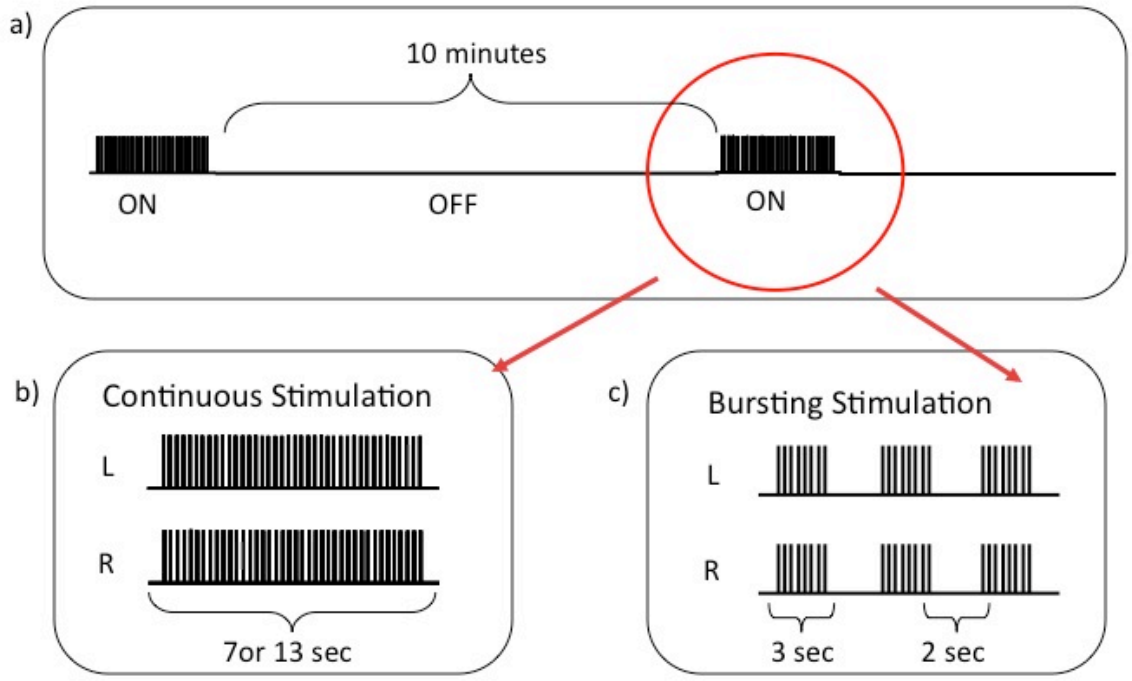

Figure 2-1: Intermittent Electrical Stimulation (IES)

IES consists of a stimulation 'ON' period of 7-13 seconds, and an 'OFF' period of 10 minutes (a). Two paradigms were tested, a Continuous stimulation (b) and Bursting stimulation (c). Within the continuous stimulation paradigm, two 'ON' durations were investigated, 7 and 13 seconds.

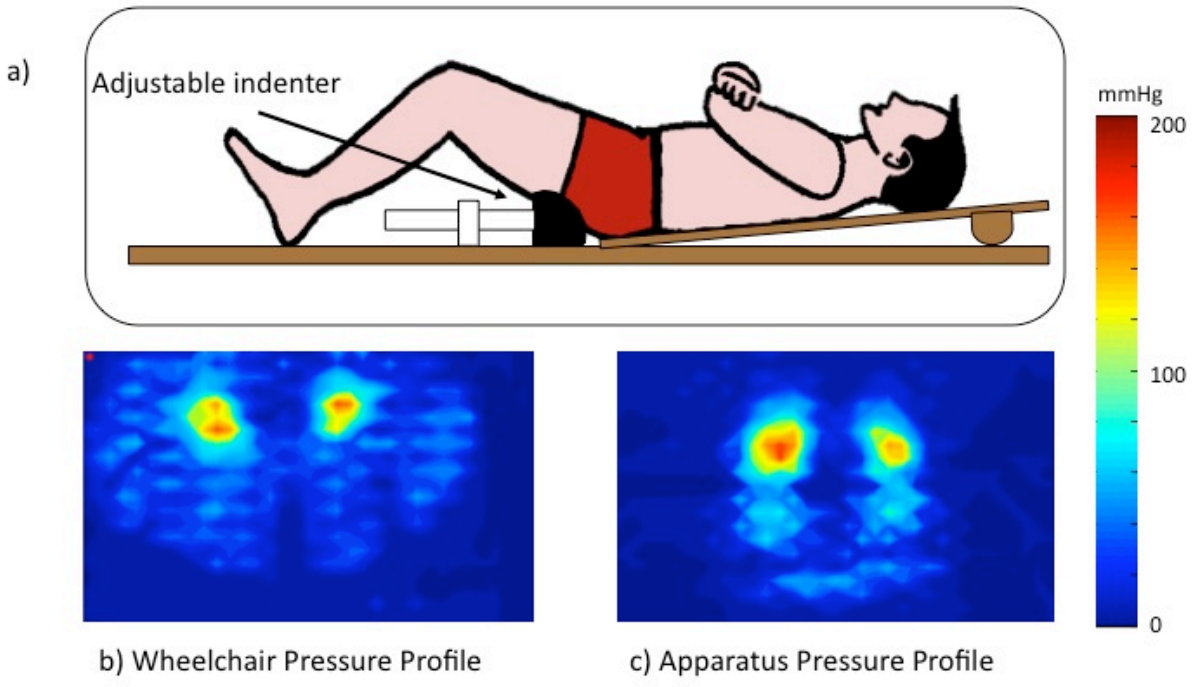

Figure 2-2: Set-up for simulating seated pressures in horizontal MR scanner

A custom-built apparatus (a) was used to simulate pre-recorded pressure profiles around the ischialtuberosities in each participant (b). The apparatus consisted of an adjustable indenter used to compress the gluteal muscles to recreate the seated pressure profile in the magnet (c).

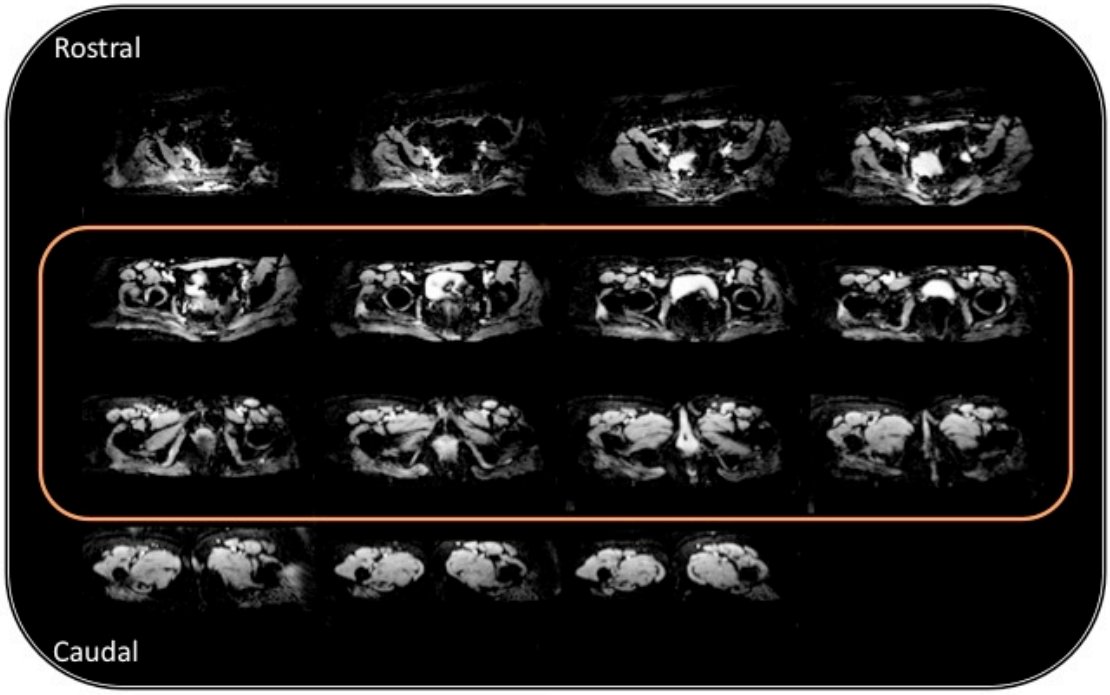

Figure 2-3: Tissue oxygenation data acquisition

15 slices per time point (as above) were obtained every 1.33 seconds over 10 minutes, for a total of 450 sets.

The 15 slices provided full coverage of the gluteus maximus muscles. Of these, 8 slices (as circled) containing the region of interest were selected for analysis.

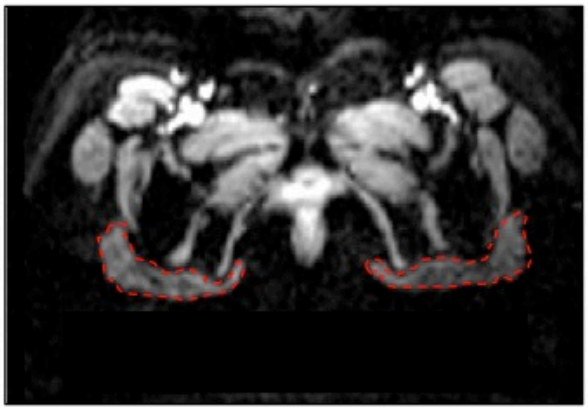

Figure 2-4: Region of interest

A region of interest (ROI) was selected around the right and left gluteus maximus muscle in each of the 8 slices. The signal intensity from the T2*-weighted imaging was determined for the time points before the 'ON' period of IES (baseline) and compared against the time point following the IES 'ON' periodto assess whether IES induced changes in oxygenationin the compressed muscle .

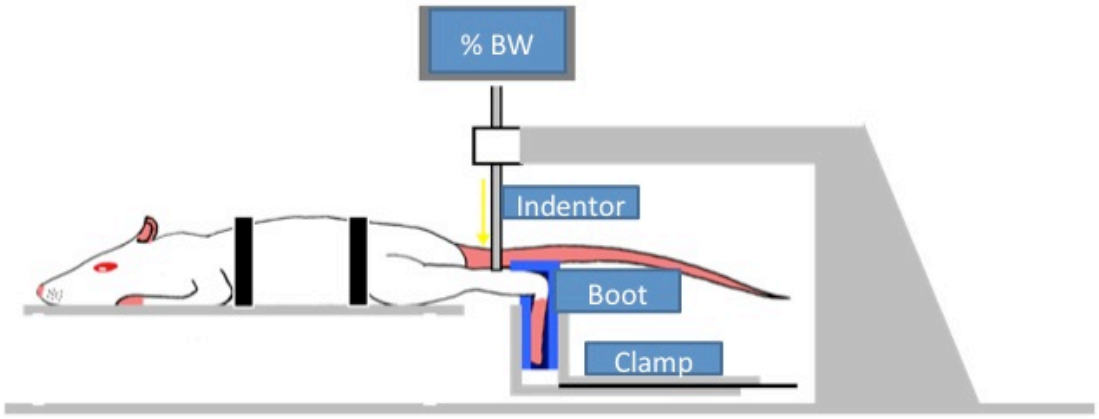

Figure 2-5: Diagram of set up for rat study

Custom-built apparatus with rat in the prone position. The foot of the experimental hind limb was rotated medially, and secured using a plaster boot and a clamp, to ensure no movement occurred during IES. A 3mm radius indenter (designed to accommodate variable loads) compressed the gastrocnemius muscle one-third the distance from the knee to ankle joint. In this model, the indenter served as the IT, and the tibia acted as the external seating surface, effectively compressing soft tissue in a similar manner to that seen in the seated condition [Solis 2007].

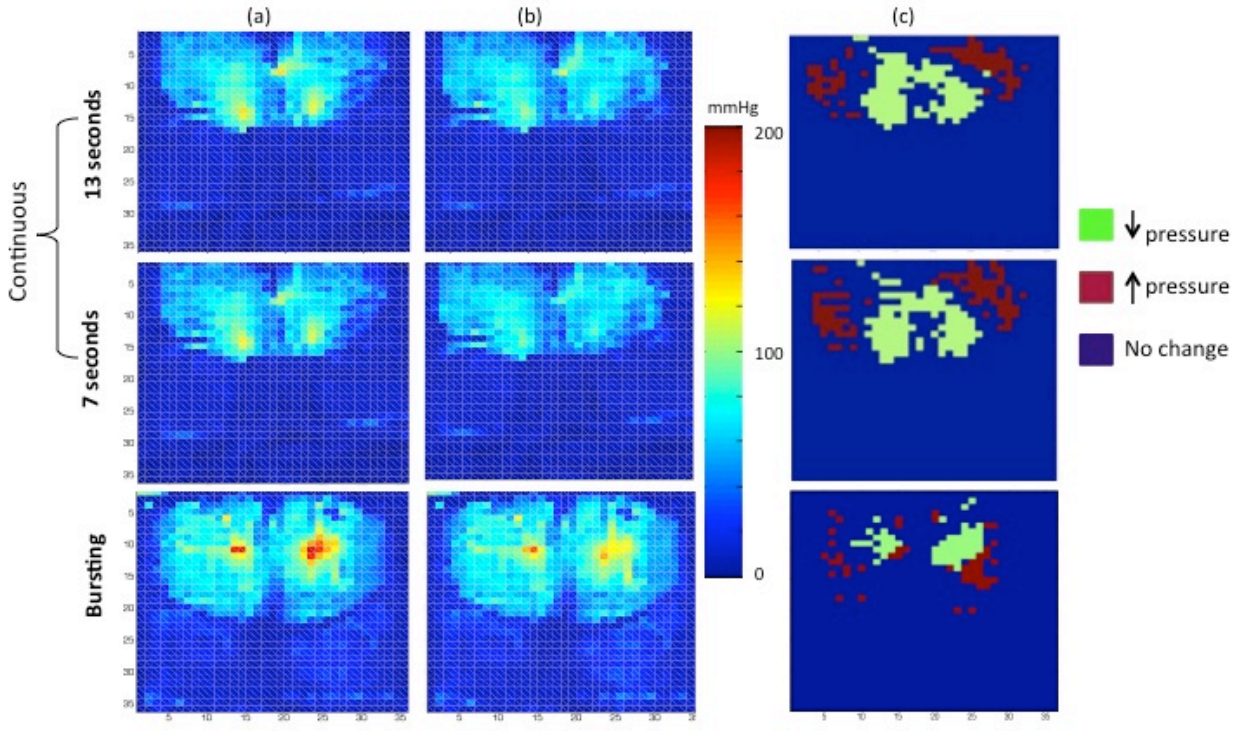

Figure 2-6: Examples of IES-induced pressure changes

Maps of pressure levels while seated on a wheelchair cushion at rest in one participant (a), during the 'ON' period of IES (b) and the location of significant pressure changes over the ischialtuberosities, induced by IES (one-way ANOVA, $p < 0.05$) relative to baseline.

The first 2 rows are from the same participant. The third row is from another participant.

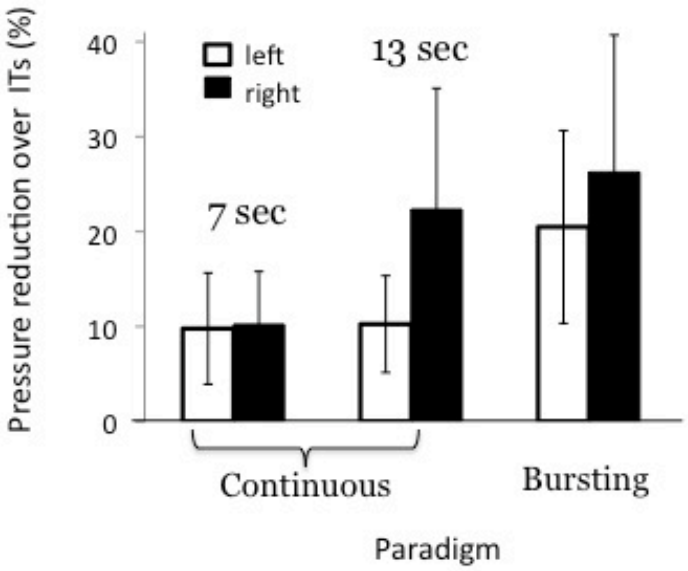

Figure 2-7: Pressure changes due to IES

Mean ± standard error representation of reduction in pressure induced by IES over areas of significant pressure reduction. (8 participants took part in both the 7 and 13 second durations of the 'ON' period of in the Continuous paradigm of IES, 7 participants in the Bursting Paradigm).

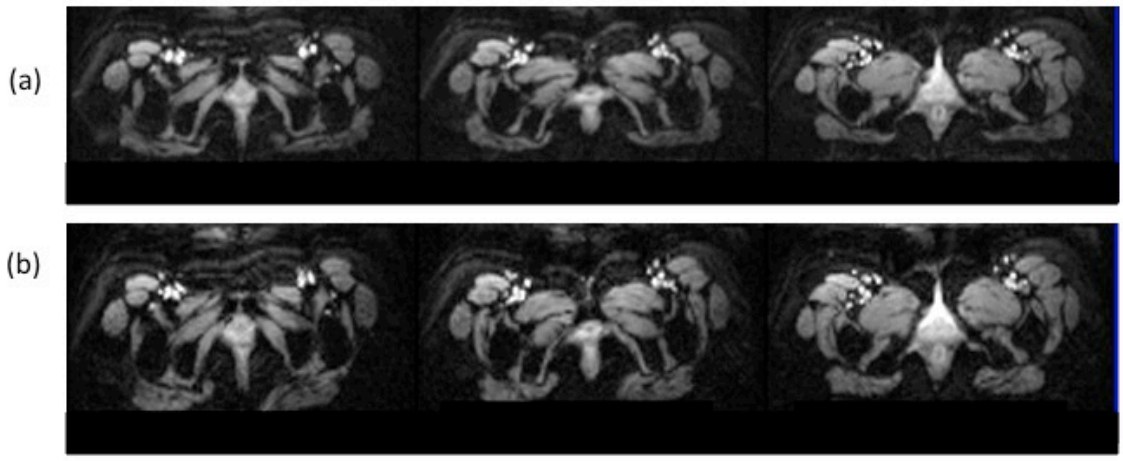

Figure 2-8: IES-induced changes in muscle shape during contraction

T2*-weighted, transverse image through hip region of a single participant. Three consecutive slices showing muscle configuration at rest (a) and the same three slices showing muscle configuration during IES -induced contraction (b).

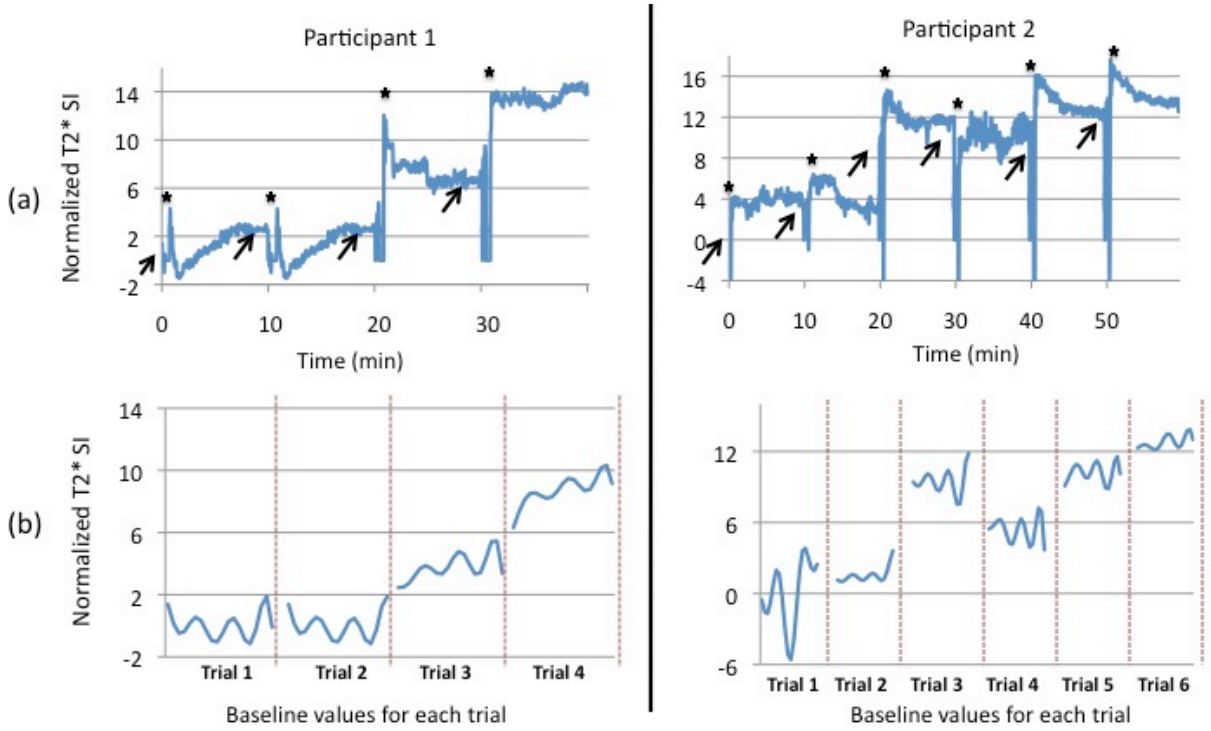

Figure 2-9: IES-induced changes in tissue oxygenation – Shifting Baseline

The changes in SI from two different volunteers during consecutive IES trials. The step-wise increases in SI following each 'ON' period of IES is demonstrated in (a). The stars denote the times the 'ON' period of IES was delivered. The large transient reductions in SI upon stimulation are due to movement artifacts produced by the muscle contractions. For each trial, the 30 second baseline SI levels before each IES 'ON' period (denoted by the arrows) are expanded (b) demonstrating the sustained and cumulative increases in baseline levels of tissue oxygenation produced by IES.

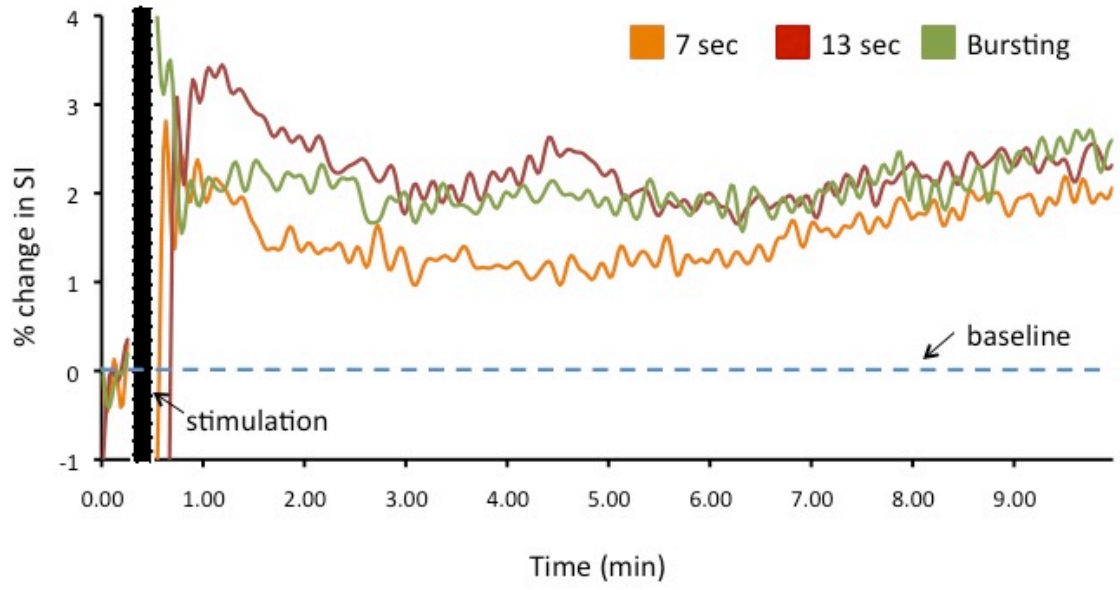

Figure 2-10 – Averaged IES induced changes in tissue oxygenation for both paradigms

Averaged data showing changes in SI, before and after the 'ON' period of IES for both paradigms. Baseline levels of oxygen and the time the 'ON' period of IES was applied are denoted.

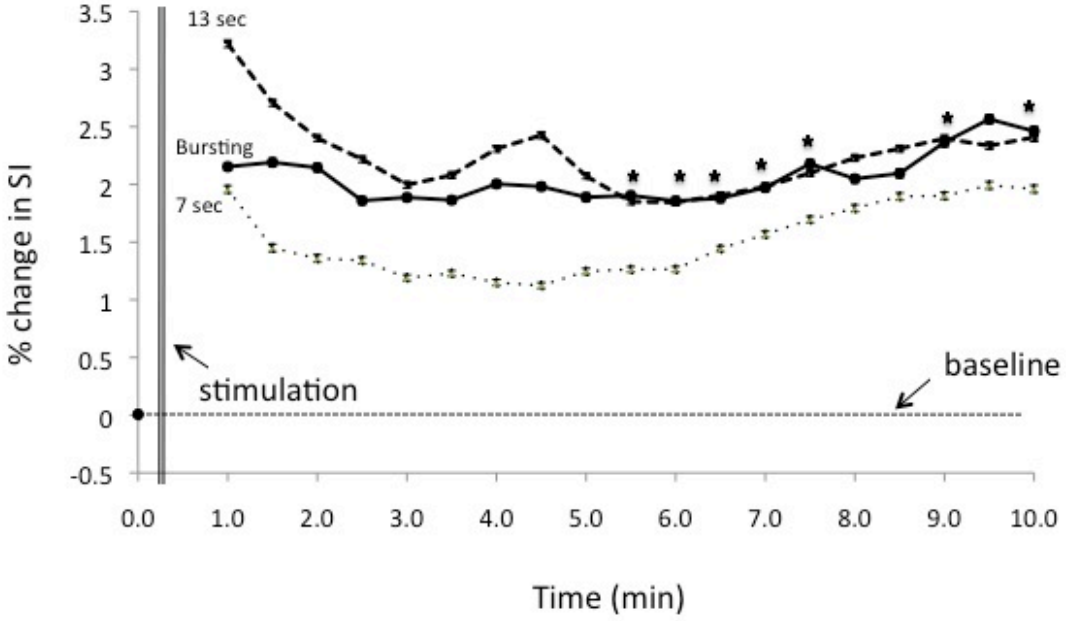

Figure 2-11 – Binned IES induced changes in tissue oxygenation

The changes in SI were binned in 30 second intervals. The mean (solid lines) and 95% confidence interval (dotted lines) are shown. The values for each paradigm was significantly elevated from baseline (one-way ANOVA, $p < 0.05$) at every time point. Each paradigm was significantly different from the other except at the points denoted with a star.

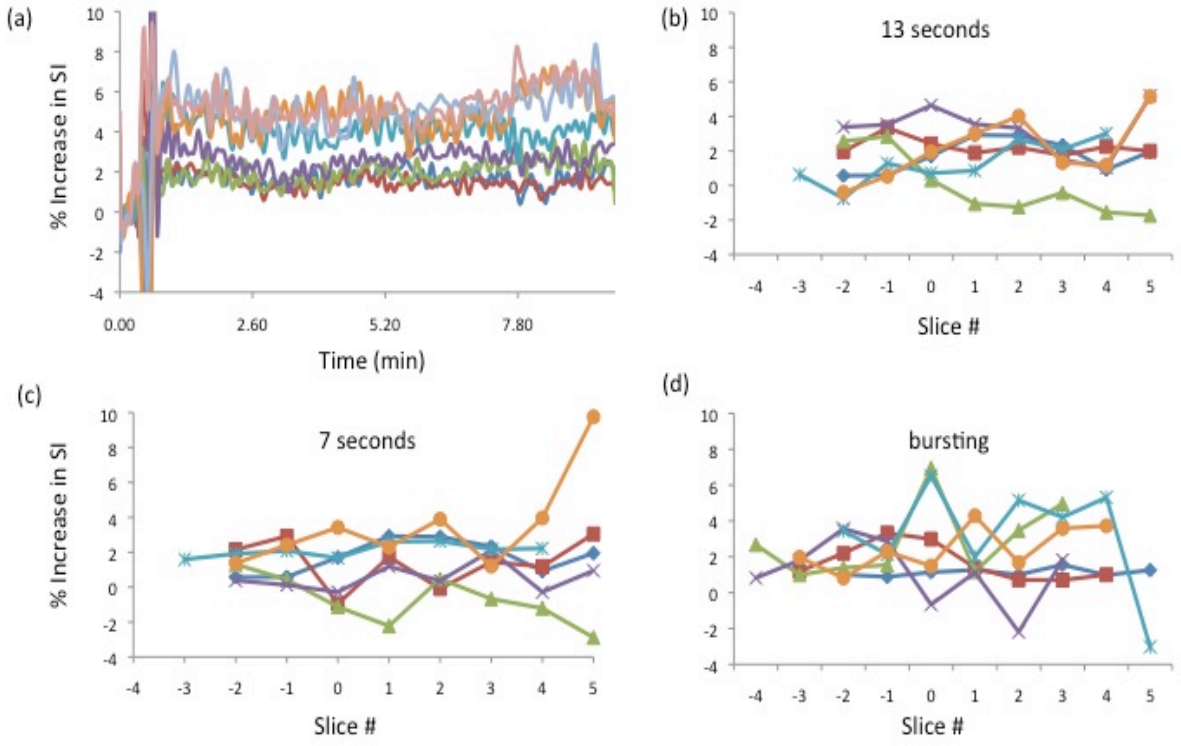

Figure 2-12: Spatial changes in tissue oxygenation

A slice-by-slice analysis was conducted to assess whether there were any spatial differences in the increases in oxygenation levels relative to the tissue most compressed by the ITs (slice 0). In (a) 8 slices from a representative sample are plotted over time. The SI values in the left and right gluteaus maximus muscles for each 8 slices were determined, and collapsed over time to represent the overall change in oxygenation in each slice. They are plotted for each paradigm, 13 seconds (b), 7 seconds (c) and bursting (d). Each line represents the normalized data for one individual. No spatial pattern was found.

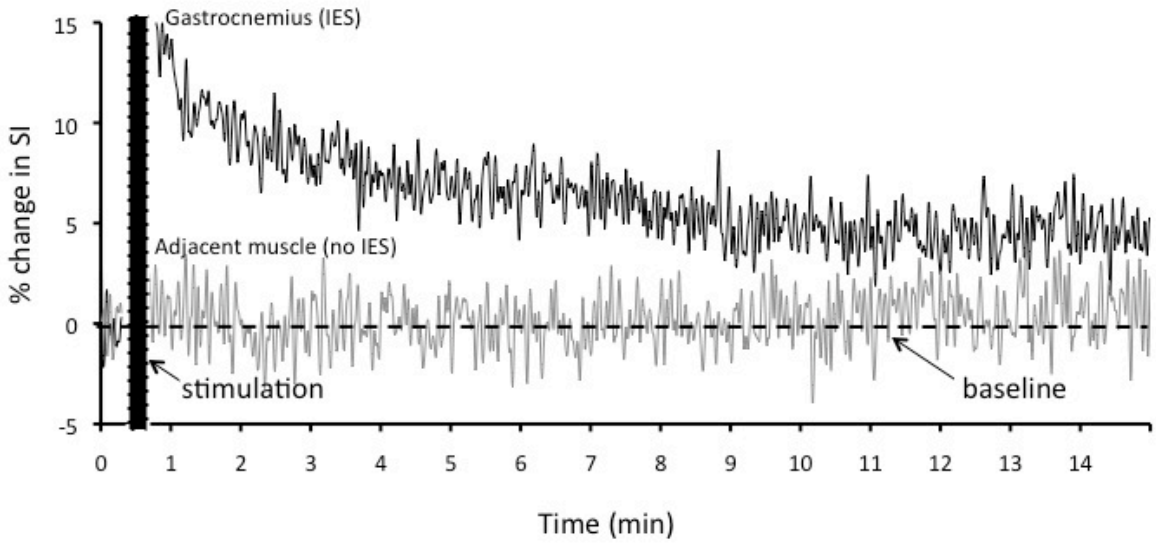

Figure 2-13: Changes in Tissue Oxygenation (Rat Study)

Raw data showing changes in SI, before and after the 'ON' duration of IES for the stimulated gastrocenemius, and an adjacent, non-stimulated muscle.

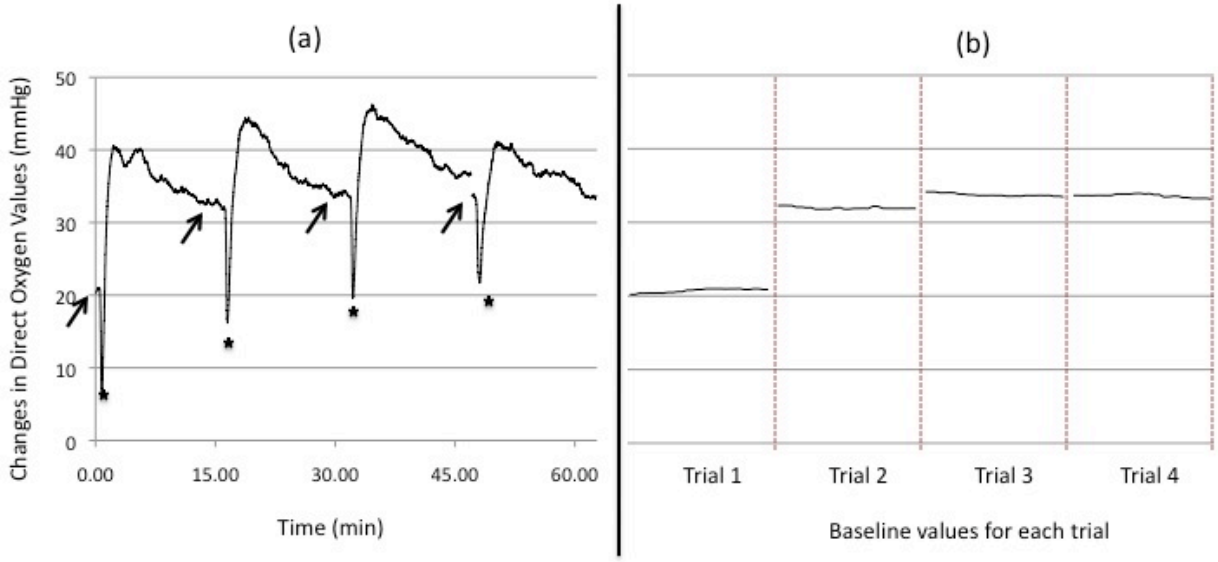

Figure 2-14: Direct oxygen measurements – shifting baseline (Rat Study)

The changes in direct oxygen measurements over 4 consecutive trials. In (a), a step-wise increase following each 'ON' period of IES, denoted by the star. For each trial, the time before the 'ON' period (baseline, denoted by arrows) is expanded in (b).

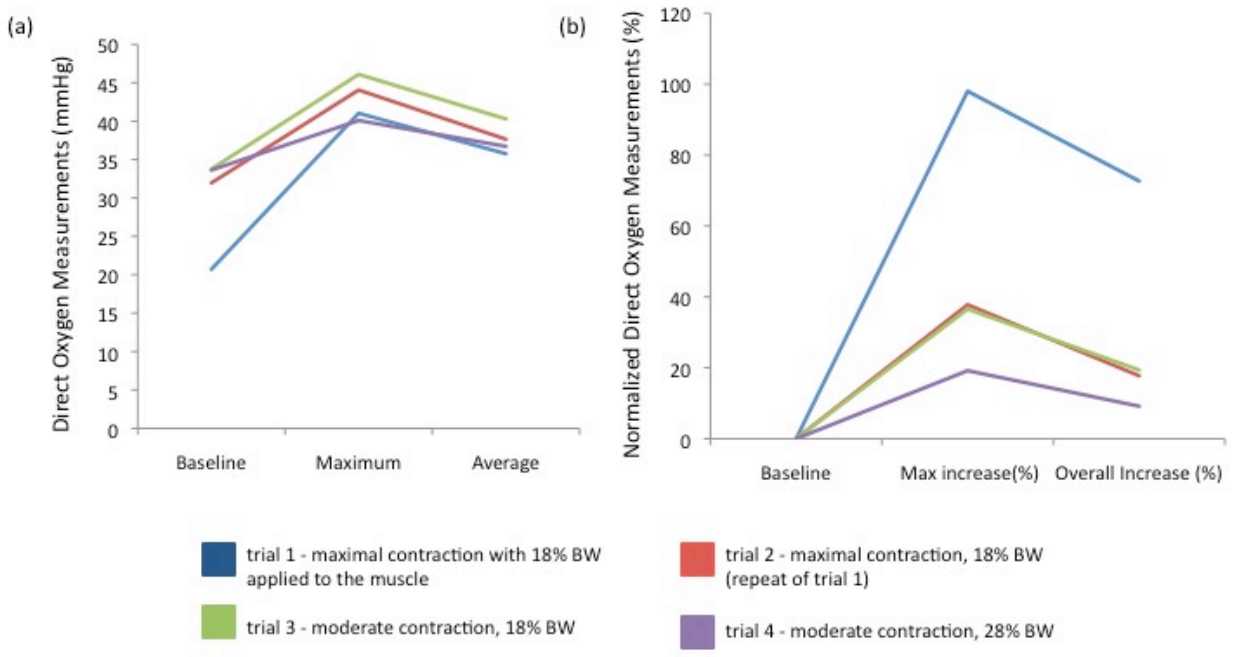

Figure 2-15: Comparison of Baseline, Maximum and Average increases in Oxygen due to IES

Results from rat study showing increases in direct oxygen measurements following IES. In (a), levels of tissue oxygenation at baseline, maximum increase, and total average increase for time values following stimulation are illustrated for each of the trials. In (b), the first 30 seconds of each trial (baseline values) were averaged, and subsequent values were normalized to these values. The percent increase for each trial, relative to its specific baseline is shown.

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Chapter 3: General Conclusion and Future Directions

The main goal of my thesis was to investigate intermittent electrical stimulation (IES) as a potential method to prevent deep tissue injury (DTI) in individuals with spinal cord injury. To achieve this, I assessed the effects of IES on two factors relevant to the formation of DTI: surface pressure, and tissue oxygenation. A pattern of significant pressure reductions over the ischial tuberosity for all paradigms was observed. IES also resulted in a significant, sustained increase in tissue oxygenation. The continuous and bursting paradigms demonstrated different patterns of increase, while the range of increase in SI was similar (2-4%). Results from the rat model of IES indicate that the increases observed in SI (from MR imaging) are not an artifact from movement, or experimental design. Furthermore, direct tissue oxygenation measurements using this model demonstrated that IES induces large increases in oxygen. The range observed following a continuous, 10 second 'ON' duration was 19-98%. This gives clinical relevance to the levels of increases observed in the human study.

3.1 Conclusions

My results indicate that IES may be able to prevent DTI by reducing pressure and increasing tissue oxygenation, thereby directly confronting the pathways of pathology that cause DTI. The results observed are similar in magnitude and time course to those seen in able-bodied individuals, indicating that the atrophied muscles and other physiological changes occurring following spinal cord injury do not dampen the beneficial effects of IES.

Based on these encouraging results, an IES system is currently under development for a long term study to test its efficacy in preventing DTI. The system is designed to take the shape of an underwear-like garment for use over the course of the day. It will initially be provided to individuals with spinal cord injury for a period of 6 months, at which point the incidence of DTI in this group will be compared to the general SCI population. If successful, the IES system could be used by any population with reduced mobility and sensation, and therefore at risk for developing DTI.

3.2 Mechanisms of action of IES

It is clear that it is the IES-induced contraction that is causing the reduction in pressure. Each contraction reconfigures the muscle shape, thus redistributing the pressure to peripheral tissues that are not as heavily loaded. The IES-induced contraction is also responsible for the increases in tissue oxygenation, though perhaps not in as direct a method as initially hypothesized (essentially, via increases in blood flow). As increases in blood flow due to reactive hyperemia or the muscle pump action are typically on the order of 10s of seconds, another mechanism must be responsible for the sustained increases in oxygenation observed in my work. One hypothesis is that the induced contraction, while affecting a transient hyperemia, also instigates a cellular level change, allowing the muscle to reduce its metabolic demands. This is especially useful in conditions such as immobilization, where muscle faces unrelieved pressure for prolonged periods of time. At its normal metabolic rate, the muscle would exhaust its available stores of oxygen and nutrients, propelling it into a state of pathology (ischemia/hypoxia). However, it appears that IES guides the muscle into a 'protective'

state, in which it requires less energy and nutrients to survive.

3.3 Future directions

The results from this thesis indicate that IES may be a viable technique for the prevention of DTI. Some limitations of the current study need to be addressed while planning future investigations:

1) Superficial pressure measurements were performed to assess the effectiveness of IES to relieve pressure in the tissue over the ischial tuberosities (ITs), for lack of a better option. However, the pressure levels at the deep bone- muscle interface are believed to be higher than those measured at the skin-surface interface [Bouten 2003; Gefen 2005; Oomens 2003; Gefen 2007; Linder-Ganz 2004]. It is therefore necessary to measure the changes in deep pressure levels due to IES to confirm that pressure reduction is occurring at the bone muscle interface, the origin of DTI. Work in an animal model of DTI with the ability to measure the changes in pressure invasively while utilizing IES throughout the soft tissue (superficial and deep) is currently underway in the Mushahwar lab.

2) The direct measurement of oxygen in the rat study provided verification and clinical relevance to the findings from the human study. However, the changes in SI and direct oxygen measurements were conducted separately. Simultaneous direct measurements of oxygen and T2*-weighted imaging while administering IES would allow for direct correlation of these measures. This is the next step in the rat study, currently underway in the Mushahwar lab.

3) In addition to the long term clinical study planned, a long term animal model could provide conclusive evidence of the efficacy of IES to prevent DTI. Previous work in the lab has demonstrated that IES can prevent damage induced when pressure is applied in a single 2 hour-long session [Solis 2007]. Using an animal model in which there is consistent loading over the course a day, and for an extended period (i.e., weeks) in the absence and presence of IES would be able to elucidate if IES is effective under more clinically relevant conditions (e.g., the compression of the gluteal muscles of individuals with SCI for hours a day, every day). It would be most realistic to carry this project in animals with atrophied muscles, or, in an animal model of SCI. Plans are underway to conduct this study in the Mushahwar lab.

4) All paradigms of IES tested (13 seconds, 7 seconds and bursting) resulted in significant increases in tissue oxygenation relative to baseline. These values were chosen in an attempt to determine the shortest contraction duration that would still result in significant increases in oxygenation. Future studies could assess the effects of even shorter durations for the 'ON' period of IES (for example: 1, 3, and 5 seconds).

5) As investigated in the preliminary rat study, there appears to be differential responses to IES in the levels of tissue oxygenation depending on the level of loading and muscle contraction strength. Interestingly, it appeared that sub-maximal contraction strengths could induce the same levels of increase in oxygenation as maximal strength contractions. These effects need to be examined in more depth to determine the boundary effects of IES.

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