The Acute Effects of Systemic Cytokines on Peripheral Nerve Function in Humans

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ABSTRACT

Cytokines have been shown to cause a reduction in nerve conduction when examined using animal models. Such effects, if shown in humans, could result in detrimental effects to physical function during periods heightened systemic cytokine concentrations. The study investigated the acute effects of cytokines on nerve conduction velocity (NCV) and functional measures. Measures were taken under both basal and elevated cytokine concentrations to determine any corresponding changes to NCV. A significant positive correlation was found between the cytokine IL-6 and NCV at 2 hours post-exercise (r=0.606, p=0.048). A significant negative correlation was found between IL-1ra and NCV at 24 hours post-exercise (r=−0.652, p=0.021). A significant positive correlation was also found between IL-1ra and endurance at 1 hour post-exercise (r=0.643, p=0.033). As such, it would seem that IL-6 may potentially act to enhance nerve function while other cytokines such as IL-1ra may have negative effects and reduce NCV.
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List of Abbreviations

ACh – Acetylcholine

APC – Antigen presenting cell

ASIA – American spinal injury association

Ca++ - Calcium

CNS – Central nervous system

ELISA – Enzyme linked immunosorbent assay

EMG – Electromyography

HRR – Heart rate reserve

IL – Interleukin

IL-1ra – Interleukin-1 receptor antagonist

K+ - Potassium

MHC – Major histocompatibility complex

mRNA – Messenger ribonucleic acid

Na+ - Sodium

NCV – Nerve Conduction Velocity

PNS – Peripheral nervous system

SCI – Spinal cord Injury

Tc – T-cytotoxic cell

Th – T-helper cell

TNF-α – Tumour necrosis factor alpha

VO_{2} – Maximal oxygen consumption
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I. INTRODUCTION

Following spinal cord injury (SCI), individuals typically regard the immediate motor and sensory losses as the largest area of concern. Loss of function below the level of the lesion produces numerous obstacles making the performance of activities of daily living and the maintenance of an independent lifestyle an arduous task. Unfortunately, the immediate functional consequences inflicting individuals following SCI are not the only issues which impact life post-injury. Numerous secondary health complications are quite common, arising due to factors such as inactivity, disuse of muscle groups below the lesion, and loss of sensation necessary in the detection of arising health issues. In turn, complications including, cardiovascular disease, muscular atrophy, urinary tract infections, and pressure sores are not uncommon among this population. The role a loss of function plays in the development of many secondary health complications is quite clear, however, it is now becoming more evident that many of these secondary health complications may in turn, indirectly affect function.

The presence of a chronic elevation in systemic cytokine concentrations has been shown to occur following trauma to the spinal cord. This chronic elevation is apparent despite a lack of any secondary health complications capable of inducing an inflammatory response. When these common health complications do arise, a resulting acute spike in cytokines occurs (Davies et al., 2007). As such, cytokines typically remain in a chronically elevated state following SCI with greatly exaggerated elevations during periods of acute inflammation. As pro-inflammatory cytokines play an important role in the inflammatory response and therefore help to aid in tissue repair, their elevation would be seemingly beneficial regarding the potential repair of damaged spinal tissue. The
presence of elevated cytokine concentrations has however been shown to be related to conduction deficits due to a phenomenon known as channelopathy. Such effects were shown in a study by Davies et al. (2006), in which the effects of tumour necrosis factor-α on the electrophysiological properties of an excised guinea pig spinal cord were examined. It was shown that elevations in the cytokine led to axonal conduction deficits, as shown by a reduction in compound action potential amplitude and a depolarization of resting membrane potential. If these same channelopathic effects were to occur in vivo, within human subjects, then cytokines, among other molecules, would have the ability to cause further detriments concerning the motor and sensory capabilities of individuals with spinal cord injuries. These effects would be of particular interest concerning individuals with some amount of spared neural tissue at the level of the lesion.

A SCI may result in the maintenance of the structural integrity of a number of spinal tracts, with a preservation of partial motor and sensory function. However, despite the fact that certain tracts remain structurally healthy, conduction deficits may still occur due to alterations in ion kinetics. Cytokines may disrupt signal conduction by interfering with the exchange of sodium (Na+) and potassium (K+) ions across the cell membrane. Through the blockage Na+ and K+ channels, a resulting disruption in membrane potential may lead to the reduction or even complete abolishment of signals. Individuals with SCI may therefore not be achieving their true motor and sensory capabilities as structurally healthy tracts may be unable to function as such.

If cytokines due in fact contribute to channelopathy then future considerations examining methods of controlling systemic levels or preventing the clogging of ion channels may help to enhance both the motor and sensory capabilities of individuals with
The purpose of the present study is to investigate the acute effects of elevated systemic cytokine concentrations on peripheral nerve function \textit{in vivo}, in human subjects.
II. REVIEW OF LITERATURE

2.1.0 Background and Epidemiology of Spinal Cord Injury

2.1.1 Anatomy of the Spinal Cord

The spinal cord provides a pivotal conduit between the brain and body in which all motor and sensory information travels. Ascending tracts carry sensory information such as touch, temperature, pain, and joint position to the brain whereas descending tracts carry motor signals to muscle groups throughout the body to initiate movement. The spinal cord is composed of outer spinal tracts known as white matter, and an inner segment comprised of neuronal cell bodies and interneurons known as grey matter. Contained within the protective sheath of the vertebral column, and surrounded by a cushion of cerebral spinal fluid, the delicate tissue of the spinal cord relays critical signals to the limbs, trunk, and organs. These signals exit and enter the spinal cord via small spinal roots which branch out through spaces known as foramina within the vertebral column. These roots then travel to their target tissue and innervate them by means of neurotransmitter release.

2.1.2 Innervation of the Spinal Cord

The spinal cord is categorized into five regions, each of which is further divided into a number of segments. Each spinal segment is responsible for innervating a unique muscle group or organ as well as receiving sensory information from a particular region of the body. Groups of muscles receiving innervation from a specific spinal segment are known as myotomes. A single spinal segment typically innervates more than one myotome, just as most muscle groups are innervated by more than one spinal segment. This overlap may have important functional implications concerning damage to the spinal
cord. If a particular region of the spinal cord is to become damaged, resulting in loss of innervation to the myotome, partial innervations may still be possible from a more rostral segment. This allows for the partial preservation of motor abilities to that muscle group. Specific areas of the skin responsible for relaying sensory information to particular areas of the spinal cord are termed dermatomes. Unlike myotomes however, dermatomes generally provide information to only one spinal segment. Due to the complex overlaying innervations in addition to varying levels, severities, and the specific location of damage to the motor neuron, spinal cord injuries (SCI) are highly variable and often differ greatly from one individual to the next.

2.1.3 Epidemiology and Demographics of SCI

The incidence rate of SCI in North America has been on the rise over the past thirty years and currently stands at 35 and 40 per million population per year in Canada and the United states respectively (Kattail et al., 2009). Of these individuals, an estimated 55% have incomplete SCI, meaning some degree of motor and/or sensory capabilities are maintained below the lesion. In regards to the level of the SCI, those to the cervical region are the most common, accounting for approximately 51% of all cases. Lower level SCI such as those affecting the thoracic and lumbar region are less common accounting for 21 and 28% respectively (Lenehan, et al., 2009).

The mean age of individuals living with SCI has traditionally been quite young at approximately 32 years of age. However, over the past thirty years an increase in the number of older adults living with SCI has become evident, due in part to advancements in treatment and care. In 2001, Sekhon and Fehlings reported an increase in SCI occurring in older adults from 4.7% in the 1970’s to 10% in 2001. The mean age in North
America is now approximated at 42.2 years of age (Pickett et al., 2006). Despite the shift in the mean age of individuals living with SCI, when concerning the age of onset, young males are still deemed to be at the highest risk and outnumber females by a ratio of 3:1 (Dryden et al., 2003).

### 2.1.4 Etiology and SCI

Etiological data suggests that the primary cause of SCI in North America is motor vehicle accidents, accounting for approximately 50% of all cases (Chester et al., 2007). This includes injuries sustained to the driver or passenger of a vehicle as well as to those hit by vehicles. Falls are regarded as the second most common cause accounting for approximately 24% of all cases. Although SCI caused by falls are only half as common as motor vehicle accidents, they are the only cause which has steadily increased over the past 30 years. Sports related injuries have shown a decline over the past 3 decades and are now responsible for only 9% of injuries. Lastly, injuries caused by violence showed a steady increase, until peaking in the mid 1990’s, however, they have since declined to an estimated 11.2%.

Based on an estimated prevalence of 755 per million population, approximately 250,000 people are currently living with a SCI in North America (Wyndaele & Wyndaele, 2006). This creates a need for further research and new treatment methods to help alleviate the physiological, psychological, and socioeconomic impact placed on individuals with SCI. As the location and severity of a SCI are highly variable from one individual to the next, no two injuries are ever exactly the same. Therefore, the development of treatment methods which can be individualized for each patient’s unique pathology is of extreme importance.
2.1.5 Injury Level

The level of spinal tissue damage will vary depending on the nature of the accident or disease and may affect one spinal segment or span over several segments. As each segment of the spinal cord is responsible for innervating a particular myotome and receiving information from a particular dermatome, the level of the injury will dictate the diagnosis of either tetraplegia or paraplegia. Tetraplegia refers to the loss or impairment of motor and/or sensory function due to a lesion in the cervical region of the spinal cord. As an injury to the cervical region is a high level injury, innervations to the upper extremities, in addition to the lower extremities, trunk, and pelvic organs will all be affected. Paraplegia refers to the loss or impairment of motor and/sensory function due to a lesion in the thoracic, lumbar, or sacral region. Due to the relatively lower level of the injury, the upper extremities remain unaffected while the degree to which the lower extremities, trunk and pelvic organs are affected vary depending on the specific spinal segment affected.

2.1.7 Severity

A SCI may widely vary in severity or the completeness of the injury. A complete injury is one in which there is no sensory or motor function at some point below the level of the lesion. However, with less severe injuries there is a greater sparing of neural tissue and improved functional outcome. Such injuries are termed incomplete and the potential for the exchange of some sensory and/or motor information remains. The completeness of an injury is determined by the presence or absence of motor and sensory capabilities in the fourth and fifth sacral regions of the spinal cord. If any amount of sensory or motor ability exists, the injury is deemed incomplete. This classification can be further specified
by considering the amount of motor and sensory capabilities preserved following SCI. By means of the American Spinal Injury Association (ASIA) impairment scale (AIS) an individual’s motor and sensory function can be evaluated based on a 5 tier scale. The scale ranges from AIS-A, denoting a complete injury, to AIS-E, signifying the individual has normal motor and sensory capabilities. A grade of B, C, or D, indicates an incomplete injury and specifies the degree to which motor and sensory capabilities are maintained (see appendix for precise definition of each classification).

2.1.8 Dyscomplete SCI

In certain cases, a SCI may be classified as complete despite the preservation of the structural integrity of a number of spinal tracts. In situations such as these, the spinal cord will have a structural appearance much like that of an incomplete injury and the preservation of partial motor and sensory function below the level of the lesion would be expected. The fact that there is an absence of function below the lesion, (specifically in the 4th and 5th sacral regions) despite a lack of structural damage to all tracts, suggests that a non-structurally based mechanism must be responsible for the apparent conduction deficits. It has been theorized that molecules seen at high levels during periods of physiological or psychological stress may be responsible. As SCI is typically characterized by high levels of each of these stressors, an elevation in stress induced molecules such as, antibodies, toxins, and cytokines may be expected.

2.2.0 Cytokines and Immunoregulatory Function

2.2.1 Cytokine Production

Cytokines are small signaling proteins involved in an array of immunoregulatory responses including hematopoiesis, and inflammation (Sredni-Kenigsbuch, 2002).
Historically, they have been viewed solely as molecules produced and released by a variety of immune cells in response to an immune stimulus. They may be produced by cells within the brain and spinal cord such as neurons, astrocytes, and oligodendrocytes, or by a variety of circulating immune cells (Davies et al., 2006). More recently, cytokines have been shown to be associated with exercising skeletal muscle. These cytokines termed "myokines" are produced by skeletal muscle and may act to help regulate glucose levels during periods of intensive exercise (Pedersen, & Febbraio, 2008). At the present time, however, far more research has been conducted on immune related cytokines, and the concept of skeletal muscles functioning as an endocrine organ is still a relatively new concept.

2.2.2 The Immune System

The immune system is responsible for protecting the body against foreign, potentially pathogenic, molecules which would otherwise cause harm through infection or disease. Human immunoregulation is a complex process characterized by two branches of immunity; the innate and adaptive systems (Medzhitov & Janeway, 1998). Each system works synergistically on both systemic and local levels to recognize, attack, and destroy foreign materials. Upon initial attack from a foreign molecule the innate system is activated. The innate system uses non-specific defenses including physical barriers such as the skin and mucous membranes, cells such as phagocytes and natural killer cells, and soluble factors such as complement to prevent infection (Janeway & Medzhitov, 2002). If the defenses of the innate system fail, the adaptive system is activated and pathogen specific defenses are used to fight off infection. A variety of lymphocytes are able to
recognize antigens by means of surface receptors and produce specific defensive proteins and antibodies to respond (Fabbri et al., 2003).

2.2.3 Innate Immunity

Within the innate immune system, cells known as phagocytes including macrophages, dendritic cells, and neutrophils, act on foreign molecules, engulfing and destroying them through a process known as phagocytosis (Janeway, & Medzhitov, 2002). Virally infected cells and cancer cells are dealt with in a different manner through a process known as apoptosis in which the cell is stimulated to undergo self-destruction. In this process, specialized cells of the innate immune system known as natural killer cells prevent viral spreading by releasing pore-forming proteins into the infected cell leading to eventual lysis (Biron et al., 1999). Natural killer cells, in addition to macrophages, are among the most prolific cytokine producers of the innate immune system and through this production, assist in the recruitment of additional immune cells during an immune response (Biron et al., 1999). These cytokines may function in autocrine or paracrine fashions and can cause a variety of responses including the up-regulation or down-regulation of the production of other cytokines, an increase in the number of a particular surface receptor, or the suppression of their own effects through feedback inhibition (Gharae-Kermani & Phan, 2001).

2.2.4 Adaptive Immunity

Immune cells known as lymphocytes, belonging to the adaptive immune system, produce specific responses to invading antigens. The adaptive immune system may be categorized into either humoral or cell mediated immunity. Humoral immunity deals with extracellular pathogens by means of circulating antibodies. When an antigen is present in
circulation, phagocytes such as macrophages, monocytes, and dendritic cells, which function as antigen presenting cells (APC’s), are able to initiate an immune response (Fabbri et al., 2003). Receptors on the surface of APC’s are activated by molecular patterns associated with a pathogen, leading to the increased expression of a protein found on the cell surface known as major histocompatibility complex 2 (MHC-2) (Cresswell, 1994). This protein is able to accept some of the foreign digested proteins of the antigen after it has been destroyed by the phagocyte. It can then present this matter to either a naïve T-lymphocytes or a memory T-lymphocyte. Naïve T-cells are a subset of T-cells which have never before encountered an antigen. Upon being presented with a new antigen a primary immune response is initiated in which the T-cell undergoes extensive proliferation within the lymph nodes producing further copies of T-cells capable of recognizing the specific antigen (Fabbriet al., 2003). Memory T-cells are those which have previously encountered the antigen, and as such, can produce a faster, stronger secondary immune response following re-exposure (Dutton et al., 1998). Upon being presented with the antigen, T-helper cells, particularly effector cells, are stimulated to release cytokines. These cytokines cause the proliferation and differentiation of B-cells into memory cells as well as immunoglobulin secreting plasma cells (Litman et al., 1993). The antibodies produced by these new plasma cells do not directly destroy antigens but instead bind to specific antigens and assist in their destruction. Upon binding to the antigen, the antibody may act to block the toxic actions of the antigen, or cause the clumping of several antigens making them more easily phagocytized (Underdown, & Schiff, 1986). In addition, antibodies activate circulating complement which coat antigens increasing the likelihood of phagocytization (Underdown, & Schiff, 1986).
Antibodies are an effective, specialized means of destroying circulating pathogens. However, they are unable to enter the cell and act intracellularly (Underdown, & Schiff, 1986). As such, if the pathogen has already entered a cell, different means of immunity are necessary.

### 2.2.5 Cell Mediated Immunity

Cell mediated immunity utilizes activated T-cells in order to attack and destroy infected host and foreign cells. T-helper cells bind to the surface of macrophages whereby MHC proteins display antigenic determinants (Cresswell, 1994). This binding causes the macrophage to release the cytokine interleukin-1 (IL-1) which stimulates the proliferation of additional T-cells. The activated T-cells also release additional cytokines known as interleukin-2 (IL-2) which cause further proliferation of T-cells including T-helper cells and T-cytotoxic (Tc) cells (Fabbri et al., 2003). These Tc cells recognize and attach to cells expressing the same antigen determinants as the original MHC protein. In a process similar to that of natural killer cells, the Tc cell releases perforin into the infected cell causing lysis (Fabbri et al., 2003). The remnants of the destroyed cell are then engulfed and cleared by phagocytes.

### 2.2.6 The Inflammatory Response

The role cytokines play in cellular communication during both innate and adaptive immune responses is an essential component of the body’s defenses. Without the increased recruitment and proliferation of necessary immune cells, the body would be unable to cope with invading pathogens and tissue damage brought on by infection would result. Although healthy individuals are generally able to fight off infection, especially
when confronted by a pathogen dealt with in the past, at times the body does succumb to tissue damage.

Whether due to infection or physical trauma, the inflammatory response is triggered in order to prevent the spreading of any pathogens, and heal damaged tissue. After the onset of trauma, vasodilators such as histamine and bradykinin are released by specific leukocytes known as mast cells and basophils. As a result, vessels are triggered to vasodilate allowing for an increase in blood flow to the site of the injury (Pober, & Cotran, 1990). This hyperemia causes an increase in the rate of delivery of different immune cells, as well as the removal of wastes and toxins. In the initial stages of the response, clotting proteins such as fibrinogens travel to the injury site and help prevent the spreading of pathogens by forming a sticky mesh in the areas adjacent to the tissue damage (Ryan & Majno, 1977). Once the pathogens are contained, phagocytic cells such as macrophages and neutrophils begin to engulf and destroy any foreign molecules. Additional phagocytes are recruited from the circulation by means of a chemical attraction to the cytokines released by local neutrophils through a process known as chemotaxis (Pober, & Cotran, 1990). As these newly recruited phagocytes travel towards the site of inflammation they are seized by the sticky membranes of local endothelial cells due to the local production of cell adhesion molecules (Meager, 1999). In addition to increased recruitment, enhanced leukocyte proliferation from red bone marrow is stimulated by the release of cytokines from activated T-cells through a process known as leukopoiesis. Together, this increase in leukocyte proliferation and recruitment aid in the inflammatory response and help return the damaged tissue to a healthy state.
2.2.7 Cytokine Homeostasis

The classification of cytokines is made extremely difficult due to their redundant and pleiotropic properties; the latter term referring to the fact that many cytokines exhibit multiple effects. They also however, demonstrate redundancy as these effects are often the same as those invoked by other cytokines (Sredni-Kenigsbuch, 2002). This causes a large degree of overlap, making the categorization of cytokines a daunting task. One such method which can be used to distinguish the properties of one cytokine from another is by their overall effect on the inflammatory process. In this sense cytokines may be deemed as either pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines act to enhance the inflammatory process by promoting leukocyte recruitment and proliferation (Hausmann, 2003). In healthy individuals an elevation in pro-inflammatory cytokines will be seen during periods of trauma or infection. These cytokines possess the ability to pass freely through the blood brain barrier and up-regulate their own expression (Hopkins, & Rothwell, 1995). As the damaged tissue is repaired and bacteria and foreign matter are engulfed and destroyed, a rise in anti-inflammatory cytokines occurs. These anti-inflammatory cytokines act as antagonists and deactivate many of the cytokine-producing immune cells to help return cytokine concentrations to basal levels (Hausmann, 2003). This cytokine balance is maintained in healthy individuals but a disruption in cytokine homeostasis has been shown to be related to several conditions including SCI.

2.3.0 Cytokines and Spinal Cord Injury

2.3.1 SCI and Cytokine concentrations

Following SCI, changes in immunoregulation have been shown to coincide with chronically elevated levels of circulating cytokines. This elevation is apparent in both the
acute and chronic stages of SCI and has been shown whether or not the individual is symptomatic for secondary health conditions (Davies et al., 2007). Varying levels and severities of SCI between individuals have been shown to have little association with the level of systemic cytokines. However, the presence of common secondary health complications such as urinary tract infections, neuropathic pain, and pressure sores have been shown to be associated with heightened elevations (Hayes et al., 2002). This has been shown to be the case with the pro-inflammatory cytokine IL-6 as well as the receptor antagonist IL-1RA, as significantly higher systemic levels were found in individuals with SCI and to an even further extent if secondary health complications were present (Davies et al., 2007). Studies by both Davies et al. (2007), and Hayes et al. (2002), also showed elevated levels of the cytokine TNF-α in subjects with SCI. However, this finding only reached statistical significance in the study performed by Hayes et al. (2002), and secondary health complications were not assessed. When examining anti-inflammatory cytokine concentrations, systemic levels of the cytokine IL-10 were not detected in either of these studies.

2.3.2 Post-SCI Immune Response

Whether immune and inflammatory responses are enhanced or suppressed following SCI depends on a number of factors including the presence of immune enhancing or suppressing cytokines, the receptor levels on glial cells, the activation status of cells, and the sequence of exposure to different cytokines (Sredni-Kenigsbuch, 2002). Some studies report immunosuppressive effects (Cruse et al., 2000) whereas others show chronic inflammation and an up-regulated immune response (Hartung et al., 1991). Knowledge regarding this effect is extremely important when considering therapeutic
approaches involving immunomodulation. The aim is to promote neuroprotection, tissue reconstruction, remyelination, and neural regeneration but if immunosuppression or autoimmune responses are to be provoked, certain treatment methods may be detrimental (Hayes et al., 2002).

2.3.3 Immunosuppression

Communication pathways exist between the nervous and immune system whereby lymphoid tissues are innervated by autonomic nerve fibers (Cruse et al., 1992). Neuroimmunoregulatory effects are therefore possible whether through a humoral response or by direct innervation of lymphoid tissue. The immunosuppression related to SCI has been found to be due, at least in part, to a disruption of sympathetic innervation of lymphoid organs (Iverson et al., 2000). Autonomic nerve fibers are responsible for innervating the lymphoid tissue of several lymphoid organs such as the spleen, thymus, and lymph nodes (Cruse et al. 2000). As these organs are responsible for the production of lymphocytes, the nervous system has the ability to directly affect immune system functioning.

Immunoregulatory function may also be influenced humorally by neurally active hormones and neurotransmitters (Carr, 1992). Lymphocytes produced by lymphoid organs have several types of hormone and neurotransmitter receptors making them prone to neuromodulatory effects (Carr, 1992). Neuroendocrine hormones may be secreted in response to the physiological and/or psychological stress associated with SCI and lead to a wide variety of immunoregulatory effects, including the immunosuppression of both the innate and adaptive immune systems. (Cruse et al., 2000).
### 2.3.4 Immune Up-regulation

There is also evidence for an up-regulated immune response following SCI due to a bias in the immune system towards a particular subset of T-cells. T-cells can be categorized into either effector T-cells or regulatory T-cells. Although overall levels stay relatively similar, after SCI there is a shift in the predominant T-cell type whereby a greater proportion of effector T-cells is evident (Davies et al., 2007). Regulatory T-cells are crucial in the maintenance of immunological tolerance and produce immunosuppressive effects through the production of anti-inflammatory cytokines (Davies et al., 2007). Effector T-cells differentiate into one of two helper T-cell subsets including T-1 helper (Th1) cells or T-2 helper (Th2) cells. Th1 cells are responsible for the promotion of cell mediated immune responses and predominantly produce pro-inflammatory cytokines such as, interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumour necrosis factor beta (TNF-β). Th2 cells promote humoral immune responses and predominantly produce anti-inflammatory cytokines such as, interleukin-4 (IL-4) and interleukin-10 (IL-10) (Sredni-Kenisgsbuch, 2002). The shift towards a Th1 cell dominance following SCI may lead to a greater proportion of pro-inflammatory cytokines. This dominance may be due to several factors including antigen quantity, and the initial APC interaction with T-cells (Boonstra et al. 2003). For example, APC’s known as dendritic cells will induce the development of Th1 cells when in the presence of high antigen levels. As individuals with SCI are prone to secondary health complications, the resulting pathogen-derived products may influence the T-helper cell response. This shift to a greater proportion of pro-inflammatory cytokines would lead to an up-regulated immune response and potentially a state of chronic inflammation.
2.3.5 *Auto-reactive T-cell Repertoire*

As secondary health complications will be associated with infection and high antigen levels, a cytokine imbalance caused by a shift to Th1 cell dominance would be expected. However, this imbalance has been shown even when no signs of secondary health complications are present. It has been proposed that this is due to a phenomenon known as the trauma induced autoreactive T-cell repertoire. The tissue damage brought on by spinal trauma is likely to release a variety of CNS proteins capable of inducing an autoimmune reaction. Over time, T-cells may begin to respond differently to certain proteins not originally associated as an antigen, leading to an immune response directed at host tissue (Popovich et al., 1996).

2.3.6 *Immune System Homeostasis*

An increase in pro-inflammatory cytokine production would have seemingly beneficial effects concerning neuronal survival and tissue repair after SCI but the disruption in immune system homeostasis has the potential to lead to immune dysfunction and immunopathogenesis. In addition, evidence exists showing that chronically elevated cytokine concentrations may contribute to axonal dysfunction (Davies et al., 2006). Therefore, it would seem that the maintenance of normal cytokine concentrations is a determining factor as to whether they will function in a positive physiologic and neuromodulatory manner, or lead to immune and axonal dysfunction as seen in a variety of neuropathies.
2.4.0 Neuropathy

2.4.1 Peripheral Nerves

Peripheral nerves consist of bundles of axons which exit and enter the central nervous system from various sites along the spinal cord and brain stem. They are categorized by the direction in which they carry signals and the function which is carried out by those signals. Afferent, or sensory fibers, receive signals from dermatomes and myotomes and carry them to the CNS, whereas, efferent, or motor fibers, conduct signals from the CNS to the target tissue. An individual axon can reach lengths of up to one meter, and as such, the ability to transmit signals rapidly while maintaining signal strength is of extreme importance. Supporting cells of the peripheral nervous system known as Schwann cells ensure the structural integrity of the neuron is maintained, and thereby act to sustain signal strength. By growing around the axon and producing several layers of lipid based protective covering known as myelin, Schwann cells act to insulate the axon. As lipids are poor conductors of electrical current, this covering provides a strong insulator to help contain travelling signals.

2.4.2 Signal Transmission

The axon is segmented into regions of myelinated and non-myelinated areas. Although myelinated areas are extremely important concerning efficient signal transmission, non-myelinated areas are necessary for the re-initiation of action potentials. Signals are typically generated at a region where the axon joins the cell body known as the axon hillock. As the signal travels along the axon it is re-initiated at non-myelinated regions known as Nodes of Ranvier. These regions are densely populated with voltage regulated gates which allow the signal to be boosted back to its original strength. This
process occurs at an extremely rapid rate and is termed saltatory conduction due to the way the signal seemingly jumps from one node to the next (Huxley, & Stampfli, 1949).

At the site of the axon hillock and each Node of Ranvier, the exchange of sodium and potassium ions causes an alteration in the resting membrane potential. As the action potential generated from the previous node travels to the next, the membrane becomes depolarized, stimulating the opening of a number of sodium channels. This allows an influx of sodium ($Na^+$) to diffuse into the cell, leading to further depolarization and stimulating the opening of additional $Na^+$ channels. If this depolarization meets the threshold of approximately -55mv, the triggering of a new action potential will result. Upon reaching threshold, $Na^+$ channels close, preventing further depolarization, and potassium ($K^+$) channels open, allowing an efflux of $K^+$ ions to diffuse out of the cell, causing repolarization and the return of resting membrane potential (Shrager, 1988).

These action potentials are non-graded, meaning that regardless of the strength of the stimulus applied, as long as the threshold is met, a standardized amplitude action potential will always be produced. These action potentials are also non-decremental meaning they maintain their strength as they travel along the axon. This is due to the constant re-initiating of the action potential at each Node of Ranvier. As the action potential arrives at the next node in the sequence the signal will be just strong enough to cause opening of the voltage gated channels and initiate a new action potential of equal strength to that of the previous node. Electrical signaling such as this is an effective communicatory pathway but can be performed only along a single continuous axon. Therefore, when an impulse must transition between two neurons, or from a neuron to a muscle fiber, such as that seen at the site of the neuromuscular junction, a different means
of signaling must be utilized. This is accomplished through chemical signaling by means of neurotransmitter release (Dulhunty, 2006).

Upon reaching the targeted skeletal muscle, the electrical impulse traveling along the pre-synaptic neuron must be converted to a chemical signal, cross the synaptic cleft, and initiate a new electrical impulse on the post-synaptic muscle fiber. This is accomplished through the release of the neurotransmitter acetylcholine (ACh) from vesicles within the pre-synaptic neuron. Upon being released, ACh crosses the synaptic cleft whereby it binds to receptors which open chemically gated channels (Dulhunty, 2006). This allows Na\(^+\) ions to flow into the muscle fiber causing depolarization of the resting membrane potential and the production of a new action potential.

The newly generated impulse then travels along specialized tubes known as transverse tubules (T-tubules) which carry the signal deep into the muscle fiber. These T-tubules transport the impulse to an internal membrane system responsible for housing calcium (Ca\(^{++}\)) ions known as the sarcoplasmic reticulum (SR). Upon stimulation of the SR, Ca\(^{++}\) ions are released into the cell (Gordon et al., 2000). This release is a crucial component in the contraction of skeletal muscle, and is required for the functioning of specialized contractile proteins. Contractile proteins including a thick filament composed of myosin and a thin filament composed of actin cause the contraction of skeletal muscle through the formation of crossbridges. Molecules of actin have binding sites for myosin which, through an energy dependent process, allow for contraction by means of a process known as the sliding filament theory. However, until stimulated by the release of Ca\(^{++}\) ions, these binding sites remain blocked by a protein known as tropomyosin. The binding of Ca\(^{++}\) ions to an associated protein known as troponin causes a conformational change.
in tropomyosin, revealing the actin binding sites and allowing for crossbridge formation (Gordon et al., 2000). The myosin heads may then perform a power stroke causing a shortening in sarcomere length, and ultimately a skeletal muscle contraction.

The above describes neuronal functioning and signal transmission under normal healthy conditions. With the support from glial cells, neurons function to send and receive both motor and sensory signals throughout all regions of the body. This system is however, extremely sensitive to any disturbances whether due to physical trauma or disease, and as such, is susceptible to a variety of neuropathies.

2.4.3 Channelopathy

Conduction deficits may also occur despite a lack of any structural degradation or demyelination. This has been shown in studies examining remission periods of individuals suffering from multiple sclerosis. These remission phases are characterized by periods of rapid recovery which occur too quickly to possibly be attributed to re-myelination (Papakostopoulos et al., 1989). Evidence exists that these non-structural based conduction deficits are caused by disorders of ion channels known as channelopathies. It has been suggested that molecules such as cytokines, antibodies, and toxins may interfere with sodium (Na\(^+\)) and potassium (K\(^+\)) channels causing increased or decreased nerve excitability (Gutmann, & Gutmann, 1996). If a Na\(^+\) channel is to become blocked, the intracellular flow of Na\(^+\) ions is reduced, making depolarization more difficult or potentially even causing complete conduction block. This will result in a reduction in action potential propagation velocity, as well as compound action potential amplitude (Gutmann, & Gutmann, 1996). The blockage of K\(^+\) channels will result in difficulty repolarizing the cell. A K\(^+\) channel block will cause a decrease in the extracellular flow of
K⁺ leading to high frequency, repetitive action potentials (Gutmann, & Gutmann, 1996). Similar effects concerning difficulty repolarizing the cell will occur if the opening of Na⁺ channels is to be prolonged. This may occur due to an immune interference leading to the prevention of channel inactivation. As a result, the neuron may become hyperexcitable leading, once again, to high frequency, repetitive action potential firing. It has been suggested that this immune interference is brought on by the demyelination experienced after nerve trauma. It is possible that the demyelination reveals epitopes which cause an antibody response against Na⁺ channels, causing a decline in the rate of inactivation (Landon, & Langley, 1971). Support for other immune constituents effecting signal conduction, such as cytokines, has also been produced.

The cytokine tumour necrosis factor alpha (TNF-α) has been shown to have both pro and anti-inflammatory effects as well as neuroprotective, neurotoxic, and neuroplastic properties (Davies et al., 2006). At normal physiological levels TNF-α has been shown to enhance synaptic transmissions through the modification of ion channel kinetics, however, at chronically elevated levels it may be cytotoxic leading to axonopathy, oligodendrogiopathy, or channelopathy (Davies et al., 2006). Tumour necrosis factor alpha may increase the intracellular flow of Na⁺ into the cell as well as the extracellular flow of K⁺ out of the cell causing enough depolarization to induce conduction failure. This accumulation of Na⁺ is also linked to increased calcium (Ca++) import which causes further disruption through the production of reactive oxygen species (Davies et al., 2006). A study by Davies et al. (2006), showed the effects of TNF-α on electrophysiological properties of an excised guinea pig spinal cord. It was shown that exposure to the cytokine caused a dose dependent and reversible depolarization of membrane potential
and a reduction in compound action potential amplitude. These effects were observed under TNF-α concentrations as low as 10ng/ml and were increasingly amplified when under higher concentrations (100, 1000, 5000ng/ml). Further evidence that it was in fact the cytokine producing the response and not the presence of other proteins was provided when exposure to heat denatured TNF-α caused no response. This study provided compelling evidence that elevations in systemic cytokine concentrations can in fact induce channelopathic effects \textit{in vitro}. These same effects however, have yet to be proven \textit{in vivo}, in humans.

\subsection*{2.4.4 Macrophage Mediated Cytotoxicity}

Non-specific tissue destruction may also occur due to macrophage mediated cytotoxicity. The release of cytotoxic cytokines and free radicals may lead to demyelination despite the fact that neither the axon nor Schwann cells are specifically targeted (Kiefer, et al., 2001). A strong inflammatory response is capable of causing such mediators to leak into untargeted surrounding tissue leading to further damage. Although this form of cytokine induced conduction deficit is structurally based, and therefore not the focus of the current study, the observation that cytokines may affect signal transmission in multiple forms is worth acknowledgement.

\subsection*{2.4.5 Neuropathy and Spinal Cord Injury}

The fact that myelinopathies and channelopathies can affect healthy, intact neurons has major implications when concerning individuals with SCI. As some of the neural tracts may be spared from the original trauma-inducing injury they have the potential to continue partially innervating tissue and relaying sensory information. However, these neuropathies may reduce or eliminate the signal production and
transmission capabilities of healthy neurons. Whether due to demyelination, alterations in ion channel kinetics, or a combination of the two, motor and sensory capabilities would become greatly reduced. As such, the true motor and sensory potential of individuals with spinal cord injury may not be met. New methods of reducing neuropathy inducing immune constituents could therefore prove invaluable to this population. A potential relationship between immune constituents and nerve function in humans was shown in a study by Mcdonald et al. in 2002. A single subject with an ASIA A, C2 level spinal cord injury was examined over a 3 year period. The individual, who was 5 years post injury, had shown no improvement in motor or sensory capabilities in the years leading up to the study. An activity based recovery program was implemented in which the participant partook in a thrice weekly, one hour bout of functional electrical stimulation cycling. Over a three year period the subject improved from the original ASIA A classification to an ASIA C classification. Interestingly, the subject’s neurological recovery increased in parallel with a decline in infection rates. These findings alone may not provide strong enough evidence to attribute a reduction in immune constituents as the sole contributor to neurologic recovery, but do however create an intriguing possible mechanism worthy of further examination.

2.5.0 The Cytokine Response to Exercise

2.5.1 Exercise and Elevations in Cytokine concentrations

Up until the early 1990’s cytokines were viewed solely as molecules involved in the regulation of immune responses. They were typically believed to be secreted by, and act on, a variety of immune cells in both paracrine and autocrine fashions. They have since been found to be secreted by non-immune cells and play a role extending far
beyond mere immunoregulation. Several cytokines, termed myokines, have been found to be secreted by contracting skeletal myocytes and produce both immunoregulatory and energy regulating effects.

Exercise induced-elevations in cytokines have been shown after periods of prolonged, strenuous physical activity. These increases are most pronounced in the pro-inflammatory cytokine interleukin-6 (IL-6), however, increases in other pro-inflammatory cytokines in addition to anti-inflammatory cytokines are also apparent (Nortoff & Berg, 1991). A study by Ostrowski et al. (1999), examined plasma pro-inflammatory and anti-inflammatory cytokines, as well as cytokine inhibitors in male subjects following a 3.5 hour marathon race. The pro-inflammatory cytokines IL-6 and TNF-alpha, as well as the anti-inflammatory cytokine IL-10 were all found to peak immediately following the cessation of exercise and increased by 128, 2.3, and 27 fold, respectively. The cytokine inhibitor IL-1RA was found to peak 1 hour after the cessation of exercise and was elevated by 39 times its resting value (Ostrowski et al., 1999). The fact that a cytokine spike may be induced by means of aerobic exercise provides the opportunity for the examination of their acute affects in a laboratory setting. Although the examination of the acute effect of cytokines following actual infection may be more ideal, predicting when and in whom an infection will occur makes it somewhat impractical in a laboratory setting. However, it has been shown that exercise-induced cytokine spikes can reach levels similar to those seen during periods of inflammation (Pedersen & Febbraio, 2008). For example, a study by Ullum et al. (1994), showed a 2 fold increase in IL-6 following 1 hour of cycling corresponding to 75% VO_{2} max. This elevation closely mimics those typically seen following SCI (Hayes et al., 2002).
2.5.2 Skeletal Muscle Damage and Cytokines

As activated monocytes are one of the primary sources of IL-6 during an inflammatory response, it was originally assumed that monocytes would be responsible for the production of IL-6 during exercise as well. The increase in plasma IL-6 found during exercise is however not matched by a change in IL-6 mRNA levels in mononuclear cells (Ullum et al., 1994). The same was shown to be true for the production of the cytokines TNF-α and IL-1β (Starkie et al., 2001). As mRNA is a precursor for, and necessary in, the production of these cytokines, it would seem that monocytes are not the source of elevated cytokine concentrations seen during exercise.

The possibility of contracting skeletal muscle as a source of exercise-induced cytokines gained attention after IL-6 mRNA was found to be increased within skeletal muscle following a period of strenuous exercise (Ostrowski et al., 1998). This increase was believed to be stimulated due to the inflammatory response brought on by the damaging of myofibres during exercise. This explanation seemed fitting due to the known increases in cytokine concentrations during immune-mediated inflammatory responses. However, a major flaw in this theory was discovered when similar elevations of cytokines were found to result when using cycling as a mode of exercise. As cycling results in only very minor levels of muscle damage it does not generally invoke an inflammatory response (Starkie et al., 2001). As such, it would seem that the inflammatory response could not possibly be the main stimulating factor associated with the exercise-induced elevation in cytokines. Further evidence for this theory was provided in a study by Croisier et al. (1998), in which subjects performed two bouts of eccentric exercise separated by a three week period. As each of the training bouts were identical, it would be expected that training adaptations would result in a much lower level of muscle
damage following the second bout, as could be shown through serum levels of myoglobin. However, despite the fact that the second exercise bout resulted in significantly lower levels of muscle damage, serum IL-6 levels were very similar (Croisier et al., 1999). Therefore, although muscle damage and the resulting inflammatory response may play a small role in cytokine elevations during and after an exercise bout, it does not appear to be the primary cause.

2.5.3 Contracting Skeletal Muscle and Cytokines

Despite providing some evidence suggesting the role of contracting skeletal muscle in the production of cytokines, the aforementioned studies offer no conclusive evidence. It is possible that the elevations in cytokines are due to other factors such as hormones, and metabolites produced during exercise which may mediate cytokine production. In order to irrefutably show that the contraction of skeletal muscle causes increased cytokine release, a study by Steensberg et al. (2000), examined the levels of cytokines and potential mediators in the exercised vs. unexercised leg. Using catheterization in the femoral artery and vein of the exercised and rested leg, the levels of IL-6 and its potential mediators were measured. It was shown that both legs had equivalent cytokine mediator levels, but that only the exercised leg released IL-6 (Steensberg et al., 2000). This provides evidence suggesting that it may be contracting skeletal muscle per se which leads to an increase in local and systemic cytokine concentrations during exercise. The main shortcoming of the technique used, is that using anterio-venous difference shows only the change in cytokine concentrations across a tissue region. The change in cytokines could therefore be the result of several possible sources including, local immune cells, adipose tissue, skeletal muscle, or bone
(Steensberg et al., 2000). Therefore, skeletal muscle cannot definitively be deemed a source of cytokines based on these results.

It was not until 2004 that strong evidence as to the cellular source of cytokines was obtained. Through the use of immunohistochemistry, in which an antibody to IL-6 was fluorescently labeled, visual observation of the amount of IL-6 and mRNA within individual myofibres was made possible. It was shown that following two hours of cycling, an increase in both IL-6 and mRNA was evident within individual myofibres in comparison to resting state (Hiscock et al., 2004). This study provided convincing evidence that contracting skeletal myocytes are in fact a source of IL-6 during exercise.

### 2.5.4 IL-6 and Energy Regulation

Inducing elevations in IL-6 is possible in-vitro by means of cell culture, or in-vivo by means of exercise. By taking individual myocytes and passively stretching them, it is possible to induce a secretion of IL-6 (Peterson & Pizza, 2009). When examining IL-6 kinetics during exercise, a general trend is seen concerning the rate of its release over the span of long duration exercise sessions. Steensberg et al. (2001), showed that over a five hour exercise period IL-6 levels remained moderately elevated for the initial three hours, but increased at a much higher rate over the remaining 2 hours. This observed change in myokine kinetics is interesting as it appears to coincide with decreasing muscle glycogen levels. If myokine release is elevated as muscle glycogen stores are depleted, it may point to an important myokine-mediated response to an energy crises. This idea gained further support after it was shown that consuming exogenous carbohydrates during an exercise bout actually reduced the systemic level of IL-6 (Nehlsen-Cannarella et al., 1997). Upon depleting muscle glycogen stores, contracting skeletal muscle must rely on blood glucose
and other substrates as energy sources. The fact that IL-6 levels seem to be inversely correlated with muscle glycogen levels would suggest that it plays a key role in fuel regulation and maintenance of blood glucose levels during exercise.

If this regulation is to occur, it would be accomplished through stimulation of the liver to increase glucose output. Increased hepatic glucose output is necessary during times of muscle glycogen depletion as the muscle will begin to utilize blood glucose for energy, thereby lowering blood sugar levels. Therefore, in order to maintain appropriate blood glucose levels, an increased release of glucose from the liver is necessary. The ability of IL-6 to stimulate hepatic glucose production was examined in a study by Febbraio et al., (2004), in which subjects received IL-6 infusion during exercise. It was shown that subjects receiving IL-6 had much higher endogenous glucose production than those working at similar intensities without IL-6 infusion. As other regulators of hepatic glucose production such as insulin, glucagon, and cortisol, did not differ between groups, it would appear that IL-6 contributes to the stimulation of hepatic glucose output.

2.6.0 Measurement of Peripheral Nerve Function

2.6.1 Electromyography and Electrode Type

Electromyography (EMG) is used to convert the electric potential generated by muscle to a visual signal which can be displayed and analyzed for the purposes of examining neuromuscular function and diagnosing potential neuromuscular disorders. By creating a visual representation by means of an oscilloscope, several physiological properties can be examined. Upon determining the time taken for an impulse to travel a given distance and cause a motor response, the speed of action potential propagation or nerve conduction velocity through the peripheral nerve can be established. It is also
possible to find the strength of the motor response through assessment of specific waveforms. Obtaining accurate measurements of electrophysiological signals can often prove quite difficult, due to numerous sources of noise, and several internal and external factors which can influence conduction. As such, proper and consistent use of instrumentation becomes crucially important in order to limit error and obtain accurate measures of electromyographical signals.

Electrode type is one such factor requiring careful consideration when performing an electromyographic assessment. Electrodes placed either on the skin, known as surface electrodes, or inserted directly into the muscle, known as indwelling electrodes, are used to collect muscle generated ionic potentials and convert them to electronic potentials through a process known as signal transduction (Drost et al., 2006). Each type has its own unique advantages and disadvantages in regards to the specificity of the signal obtained and the associated noise. Surface electrodes termed “floating electrodes” are placed over the skin on top of a thin layer of electrolyte gel. They are advantageous in the sense that they provide a non-invasive, relatively simple method of obtaining signals, but also have several shortcomings. The fact that the electrodes are placed over a layer of gel makes them susceptible to a form of noise known as motion artifact due to changes in gel thickness (Talhouet & Webster, 1996). In addition, although receiving signals from some distance away from the muscle allows for the obtainment of information about the “big picture”, obtaining highly specified signals is not possible. This distance also makes them susceptible to a phenomenon known as crosstalk in which signals from muscles unrelated to those of interest, pollute the desired signal. As such, obtaining clear signals from deeper and/or smaller muscles becomes quite difficult (Bogey et al., 2000). When highly
specified signals are desired, indwelling electrodes may be more appropriate. Although more invasive, indwelling electrodes have the ability to detect individual motor unit action potentials and can be placed directly into the muscle allowing for signal recordings of deeper muscle groups (Bogey et al., 2000). Whether a surface or indwelling electrode is more appropriate for a given test will therefore depend on the location of the muscle group in question, as well as the desired specificity of the signal.

2.6.2 Electrode Configuration

Both the configuration and placement of electrodes are crucial aspects of accurate recordings. Electrodes are most commonly placed in one of either a monopolar or bipolar configuration. A monopolar configuration uses one electrode to record muscle activity while a second and third act as the reference and ground. In a bipolar configuration two recording electrodes are placed over the muscle. The placement of two recording electrodes over the muscle makes a bipolar configuration more effective concerning noise reduction. As the signal will be obtained twice, the subtraction of one signal from the other makes it possible to eliminate much of the unwanted noise while maintaining the true signal. However, when determining nerve conduction velocity (NCV) a monopolar configuration is more appropriate. As the determination of NCV is based on the time period between initial stimulation of the nerve and the invoked motor response, the use of two electrodes will result in a less accurate measurement. This is due to the fact that the time taken for the impulse to travel from the first to second electrode will affect the calculated latency period, resulting in a slower calculated NCV. As such, the use of a monopolar configuration will result in more accurate results as the latency period will be
based only on the time taken for the impulse to travel from the point of stimulation to the
motor point of the muscle.

Proper and consistent placement of electrodes as well as point of stimulation is
also very important when concerning the obtainment of precise measures. In order to
achieve the most accurate signal, the recording electrode must be placed directly over the
motor point. The motor point is a region in which the lowest level of electrical
stimulation will result in a muscle twitch (Roy, et al., 1986). As such, by stimulating
different areas of the muscle with a very low voltage, the motor point can be located. As
a monopolar configuration uses only one recording electrode, proper inter-electrode
distance does not apply. However, it is necessary to keep the distance between the point
of stimulation and the recording electrode consistent between testing. Any changes in this
distance will result in variations in the calculated nerve conduction velocity which may
be falsely attributed to fiber characteristics.

2.6.3 Signal Amplification and Filtering

Upon being converted through signal transduction, the small electronic potentials
travel to an amplifier whereby the magnitude is increased to a level large enough to be
recorded and analyzed. The amplifier also acts as a filter, removing unwanted noise
through a process known as differential gain. As the electrodes are placed at some
distance apart, the action potential will not be detected at exactly the same time.
Therefore, any signal that is detected at exactly the same time is considered noise known
as “common mode” and can be eliminated. The complete elimination of noise is not
possible due to the fact that common mode signals will never be completely identical due
to differing electrode-skin impedances. Amplifiers have however been shown to be quite
effective at removing noise which can be shown through a measure of effectiveness known as the common mode rejection ratio. Additionally, amplifiers possess the ability to filter out signals of specific frequencies. High frequency filters act to block any low frequency signals allowing only high frequency signals through, while low pass filters act in an opposite fashion. Band pass filters can also be used in which both high and low frequency signals can be excluded, leaving only those signals in the middle frequency range. Each of these filters can be used to eliminate unwanted signals while maintaining signals within a particular range of interest.

2.6.4 Electromyographic Waveforms

Upon being filtered and amplified, the analog EMG signal is either displayed directly onto an oscilloscope or converted into a digital waveform by means of an analog-to-digital conversion board. As an oscilloscope displays latency periods and wave amplitudes, it is sufficient when determining nerve conduction velocity, and the strength of the motor response. An invoked potential will produce a standardized set of waveforms which can be interpreted to provide information about a number of electrophysiological responses. When invoking a potential, the stimulator is oriented such that the cathode faces the intended target. Therefore, if the invoking of a direct motor response is desired, the cathode will face the targeted muscle group. Immediately following initial stimulation the oscilloscope displays a short duration spike known as the shock artifact. The waveform is followed by a period of relative electrical silence until the occurrence of a wave of larger amplitude and longer duration. This wave is known as the M-wave and represents the motor response of the muscle. The time between the shock artifact and the M-wave therefore represents the amount of time taken for the
impulse to travel along the peripheral nerve before reaching the muscle. This time phase is termed the latency period and can be used in the calculation of NCV. Simply by using this information in conjunction with the distance the impulse traveled, the NCV can be calculated. The amplitude of the M-wave also provides important information about the strength of the motor response. It is positively related to the strength of the applied stimulus and can provide information about motor unit recruitment. For example, the lowest possible stimulus which will invoke a twitch represents one motor unit, whereas a supramaximal stimulus represents the recruitment of all motor units. This may be used as a methodological control, making certain that the same numbers of motor units are always recruited and ensuring that any changes seen are due to actual physiological responses and not simply due to methodological errors.

The strength of the applied stimulus will also affect which type of nerve is stimulated and the resulting waveforms produced. As motor neurons are much smaller in diameter than sensory neurons they produce a greater resistance to travelling signals. Therefore, a larger stimulus is required in order to directly stimulate alpha motor neurons than that required to stimulate 1a sensory neurons. As such, if the stimulation is not strong enough, only sensory fibers will be stimulated and the amplitude of the M-wave may be significantly reduced or abolished. In this case, a delayed waveform known as the H-reflex occurs. This delay occurs due to the fact that sensory fibers carry the signal back to the spinal cord before relaying the message to the muscle through motor fibers. As stimulation is increased, alpha motor neurons will eventually be stimulated resulting in an M-wave. If this stimulation continues to be increased, antidromically propagating signals of the M-wave will begin to travel away from the muscle, back towards the spinal cord.
causing a collision with the orthodromically propagating signals of the H-reflex. As the M-wave amplitude increases, the amplitude of the H-reflex will begin to proportionally decline until it is completely abolished. Although the H-reflex is not the waveform of interest when examining nerve conduction velocity, the ability to differentiate between it and the M-wave is critically important concerning accurate measurement.

2.7.0 Summary

As cytokines have been shown to be at elevated levels following SCI it is important to determine whether the channelopathic affects seen in animal models also occurs in humans. The discovery of skeletal muscle as an endocrine organ allows for the manipulation of systemic cytokine concentrations, providing the unique opportunity to examine such effects in humans in vivo. The elevations in myokines observed following periods of strenuous exercise have been shown to be comparable to levels during periods of inflammation, making it an appropriate method of examining the acute effects on peripheral nerve function. Should cytokines be shown to negatively influence nerve function, the implications regarding individuals with spared neural tracts could prove invaluable. Future studies may begin to examine methods of reducing systemic cytokine concentrations, or preventing ion channel disruption. This would allow spared neural tracts to begin affectively participating in signal transmission thereby returning partial motor and/or sensory function.
III. Purpose and Hypothesis

3.1.0 Statement of Purpose

The purpose of this study is to determine the acute effects of exercise-induced elevations in cytokines on human peripheral nerve function in vivo.

3.2.0 Hypothesis

It was hypothesized that an elevation in systemic cytokine concentrations would lead to conduction deficits due to the proposed channelopathic effects of cytokines. Specifically, one hour of intense aerobic exercise was expected to increase each of the cytokines of interest to at least two-fold its original resting values. In terms of peripheral nerve function, it was expected that post nerve conduction velocity tests would show a decline in the speed of action potential propagation in comparison to pre-intervention values. Conduction deficits were also expected to lead to decreased post-intervention values in both muscular strength and endurance. Lastly, the time taken to perform a finger dexterity test was expected to increase. Overall, upon completion of the exercise intervention, an elevation in systemic cytokine concentrations is expected to have detrimental effects concerning peripheral nerve function, and as such, produce negative affects concerning each of the functional measures.
IV. Methodology

4.1.0 Subjects

Twelve healthy, able-bodied, college-aged males from Brock University were recruited for participation in this study. Participants were all between the ages of 20-24 and had an average age of 23.3 years. Participant weights varied from 67.9-96.5kg with an average weight of 80.9kg. All participants were recreationally active, but did not participate in competitive sports at an elite or varsity level. All participants were healthy, with no signs of neuromuscular disease or contraindications to vigorous physical activity, as ensured through the completion of a PAR-Q form prior to participation. Any signs of these conditions would have resulted in the exclusion of the individual from the study. Upon completion of the PAR-Q form informed consent was obtained from all subjects. All subjects were informed of their right to decline participation in the study or withdraw at any time without penalty.

4.2.0 Exercise Intervention Protocol

The exercise intervention consisted of 2 bouts of cycling for a duration of 1 hour per session. The chosen mode and duration of the intervention was deemed the most appropriate method of inducing a cytokine spike as exercise involving large muscle groups, and occurring over longer durations has been shown to lead to the largest increase in systemic cytokines (Pederson & Febrasio, 2008). The first bout was performed on Day 1 during the familiarization period in order to determine the appropriate relative intensity each subject would be working at on Day 2, as well as to determine fluid loss. By calculating each subjects target heart rate it was possible to set an intensity that would ensure all subjects were working at a high yet sustainable rate. All subjects performed the
cycle at an intensity corresponding to approximately 65% VO$_{2\text{max}}$. This intensity was monitored during exercise through the use of heart rate reserve (HRR), as it has been shown to be highly correlated ($r = 0.990$) with %VO$_{2\text{max}}$ (Swain et al. 1998). By determining each subject’s maximum heart rate and resting heart rate it was possible to determine HRR and target heart. Each subject was then told their target heart rate corresponding to 65% HRR. Percentage HRR has been shown to be an accurate predictor of intensity and ensured all participants cycled at the same relative, high yet sustainable intensity. At the midpoint (30 min) of the initial exercise bout, the wattage and RPM each subject was working at in order to maintain their target heart rate was recorded. This wattage and RPM was to be used as a measure of intensity for the exercise intervention on Day 2. The use of wattage and RPM as opposed to heart rate during the exercise intervention allowed for a more consistent means of monitoring intensity as cardiac drift was not a confounding factor. As hydration levels can greatly affect measures of nerve function, fluid loss over the initial bout of exercise was determined. Each subject was weighed prior to and following the exercise bout and fluid loss was estimated based on weight loss (see table #1). The estimated fluid loss was then used as a means of determining necessary fluid intake to be consumed during the exercise intervention on Day 2. The exercise intervention on Day 2 was performed a minimum of 72 hours after the familiarization session to provide an adequate time period for any elevations in cytokine concentrations to return to basal values prior to measuring resting cytokine concentrations as seen during baseline testing. During the exercise bout subjects consumed water at 6 time points (every 10 minutes) over the span of the 1 hour exercise intervention in order to ensure hydration levels were maintained. As nerve conduction
velocity has been shown to be effected, not only by cytokines, but by exercise related factors such as metabolites, temperature, and hydration levels, a method of controlling for these factors was necessary. Local metabolite production and temperature change were controlled for by assessing all outcome measures in the inactive left arm as opposed to taking measures at a location near the active muscles of the legs. Lastly, as all measures were taken 30 minutes after the cessation of exercise, enough time was provided for any elevations in temperature to return to normal. The return to temperatures close to that of pre-exercise levels was insured by monitoring skin temperature by means of a surface temperature thermometer utilized prior to all pre and post-exercise measures (see table #1). Each exercise session began with a 5 minute warm up period and ended in a 5 minute cool-down period in which the subject was instructed to cycle at a light intensity of their choosing. All subjects were required to maintain the set intensity and complete the 1 hour cycle in its entirety, or their results were to be excluded from the study.

Table #1: Subject Temperature and Weight prior to and following exercise bout

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre-exercise Temp (°C)</th>
<th>Temp 1h Post (°C)</th>
<th>Temp 2h Post (°C)</th>
<th>Temp 2h Post (°C)</th>
<th>Pre-Exercise Weight (kg)</th>
<th>Post Exercise Weight (kg)</th>
</tr>
</thead>
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<td>28.2</td>
<td>31</td>
<td>94.94</td>
<td>94.1</td>
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<tr>
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<td>32.8</td>
<td>33.1</td>
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<td>80.9</td>
</tr>
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<td>31.3</td>
<td>80.1</td>
<td>78.4</td>
</tr>
</tbody>
</table>
4.3.0 Outcome Measures

All outcome measures were taken both before and after the exercise intervention to determine any changes in each of the testing variables under basal cytokine concentrations in comparison to elevated cytokine concentrations. The first assessment of nerve conduction velocity (NCV) was performed on Day 1 during the familiarization session. On Day 2, baseline testing was performed prior to the exercise intervention and included an assessment of NCV, functional measures, and cytokine concentrations. Blood draws were taken for the analysis of cytokine concentrations, followed immediately by NCV testing and the remaining functional measures. The first blood draw was taken 30 minutes after the cessation of exercise in order to allow adequate time for each of the cytokines of interest to reach elevated levels (Ostrowski et al., 1999). Post testing of each measure, with the exception of cytokine concentrations, was also repeated at 1 hour, 2 hours, and 24 hours post-exercise in the same fashion as baseline testing. Measures at multiple time points were necessary due to the potential delay of channelopathic effects.

4.3.1 Cytokine Concentrations

Cytokine concentrations were determined through the obtainment of blood draws from the antecubital vein of each subject. A total of 10ml of blood was taken at both the pre and post testing periods. The cytokines of interest included interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor-α (TNF-α), and interleukin-1 receptor antagonist (IL-1RA). As different cytokines reach peak levels at unique times following exercise, it was necessary to perform post-exercise blood draws at an appropriate time to ensure each cytokine of interest was adequately elevated. The cytokines IL-6, IL-10, and TNF-α have all been shown to peak immediately following the cessation of exercise,
whereas the receptor antagonist IL-1RA has been shown to peak 1 hour after the cessation of exercise (Ostrowski et al., 1999). As both IL-6 and IL-10 levels begin to decline rapidly immediately after peaking, it was necessary to obtain the blood draws at 30 minutes following the cessation of exercise. Upon obtaining the blood draws, the blood was allowed to clot for 20 minutes. The serum was separated from the whole blood and frozen at a temperature of -80°C until the serum cytokine analysis was performed via enzyme-linked immunosorbent assay (ELISA). ELISA kits for each of the cytokines were provided by R&D systems, Minneapolis, U.S.A.

4.3.2 Nerve Conduction Velocity

Action potential propagation velocity as well as M-wave amplitude was determined by means of a nerve conduction velocity test. Prior to performing the test, the electrode locations were prepared by shaving the skin, removing any dead skin cells with an abrasive gel, and disinfecting the areas with rubbing alcohol. A surface electrode was then coated in an electrolyte gel to create a “bridge” between the electrode and skin surface and placed over the motor point of the flexor pollicis brevis. A ground electrode was placed over the electrically neutral location of the palm of the hand. The median nerve was then stimulated at 2 separate locations at known distances from the recording electrode. The median nerve was stimulated at a distal location at the wrist between the flexor tendons of the hand. A proximal location was also stimulated at a site just rostral to the elbow on the medial side of the biceps brachii. By recording the time taken between the initial stimulation and the motor response of the flexor pollicis brevis, and using it in conjunction with the known distance traveled, nerve conduction velocity between the two stimulation points could be calculated.
When electrically evoking an action potential, a standardized set of waveforms are displayed by means of an oscilloscope. A short duration spike known as the shock artifact represents the initial stimulation of the nerve and a longer duration wave known as the M-wave represents the motor response. Therefore, through this visual representation of the signal, the time between these two waveforms allows for the calculation of the time taken for the impulse to travel the length of the nerve and provides the information necessary in the calculation of NCV. A total of 10 trials were taken and averaged for each stimulation site. An algorithm was then utilized to accurately identify the end of the shock artifact and beginning of the M-wave to be used in the calculation of NCV. All data concerning nerve conduction velocity was analyzed via Matlab.

![Figure 1: Distal and Proximal Waveforms (sample data from subject 2 / testing day 2)](image)

**4.3.3 Muscular Strength**

A hand dynamometer was used to assess muscular strength. Each subject was asked to perform a maximal voluntary contraction by gripping the dynamometer as tightly as possible. Force production was recorded, and the highest peak force was taken as an indicator of muscular strength (Nicolay & Walker, 2005).
4.3.4 Muscular Endurance

Muscular endurance was also assessed using a hand dynamometer. Each subject was asked to maintain a maximum voluntary contraction for 30 seconds. The mean force production obtained from the final 5 seconds of the test was then divided by the mean force production obtained from the initial 5 seconds of the test. The value obtained was taken as an indicator of muscular endurance in which higher values, approaching a score of 1, represented greater endurance and fatigue resistance (Nicolay & Walker, 2005).

4.3.5 Finger Dexterity

Two tests were used to determine the level of finger dexterity. The first of which was a finger tapping test developed by Kapandji et al. (1986). Subjects were asked to perform a predetermined sequence in which the thumb was brought to different regions of the four fingers. The sequence consisted of: touching the lateral side of the second phalanx of the index finger, the lateral side of the third phalanx of the index finger, the tip of the index finger, the tip of the middle finger, the tip of the ring finger, the tip of the little finger, the DIP crease of the little finger, the PIP crease of the little finger, the proximal crease of the little finger, and finally the distal volar crease of the hand (Kapandji, 1986). The time taken to complete an errorless sequence was then recorded as an indicator of finger dexterity (see image #2). Subjects then performed a dexterity test utilizing the Purdue Pegboard. Each participant was given 30 seconds to place as many pegs into the pegboard as possible using only their left hand. The pegs were to be picked up and placed one at a time, and the number of pegs successfully placed was used as an indicator of dexterity (see image #3).
4.4.0 Data Analysis

All statistics were calculated using Excel and Statistica. The change in cytokine concentrations prior to and following the single bout of treadmill exercise was determined using a Student’s t-test. The change in electrophysiological measures, as well as the change in grip strength, endurance and finger dexterity was determined via 1-way repeated measures ANOVA (with 4 levels for time; pre-exercise as well as 60min, 120 min, 24hr post-exercise). Pearson’s r correlation analysis was used to determine relationships between exercise-induced changes in cytokine concentrations and changes in function as well as changes in NCV. Statistical significance was set at $p \leq 0.05$ for all statistical analyses.
V. Results

5.1.0 Exercise Performance

All subjects successfully completed the 1 hour cycle in its entirety with no adverse events. Fluid levels were maintained based on the previously calculated estimated fluid loss (see table #1). Following exercise, adequate time was provided for body temperature to return to similar levels as seen during initial baseline testing (see table #1).

5.2.0 Cytokine concentrations

A Student’s t-test was used to determine if cytokine concentrations were significantly different prior to and following a single bout of aerobic exercise by means of cycling. Interleukin-10 levels underwent a significant elevation from an average resting value of 10.94 ±10.25 pg/ml to an average post-exercise value of 27.52 ± 13.58 pg/ml (p=0.001, Figure 1a). Interleukin-1 receptor antagonist was significantly elevated with an average resting value of 121.77 ±37.04 pg/ml and an average post-exercise value of 210.88 ± 157.52 pg/ml (p=0.051, Figure 1b). Tumour necrosis factor alpha reached significantly elevated levels with an average resting value of 5.74 ±5.32 pg/ml and an average post-exercise value of 15.99 ±15.89 pg/ml (p=0.031, Figure 1c). Interleukin-6 was significantly elevated from an average resting value of 6.70 ±7.84 pg/ml to an average post-exercise value of 14.27 ±7.14 pg/ml (p=0.018, Figure 1d).

5.3.0 Nerve Conduction Velocity

A repeated measures ANOVA was used to determine if there was a main effect for time across four time points including baseline testing, 1 hour post-exercise, 2 hours post-exercise, and 24 hours post-exercise. No significant effect was found (F=2.55,
p=0.054, Figure 2) and therefore nerve conduction values obtained during baseline testing were not found to be significantly different from 1 hour post values, 2 hour post values, or 24 hour post values.

5.4.0 Functional Measures

A one way repeated measures ANOVA was used to determine if there was a main effect for time across each of the four time points for peak force production, endurance, and dexterity. No significant main effect was found for peak force production (F=0.437, p=0.728, Figure 3), endurance (F=0.841, p=0.482, Figure 4), or dexterity as measured by the Kapandji finger tapping test (F=0.636, p=0.598 Figure 5). When measured by means of the Purdue pegboard test, a significant main effect for time was found (F=5.15, p=0.005, Figure 6). A Tukey’s post analysis revealed significant differences between cytokine concentrations at baseline compared to those at 2 hour post-exercise (p=0.004) and between cytokine concentrations at 2 hours post-exercise compared to those at 24 hours post-exercise (p=0.048).

5.5.0 Correlations

5.5.1 Interleukin-6 & Nerve Conduction Velocity

Pearson’s r correlations were performed to determine if any correlation existed between absolute changes in IL-6 concentrations and nerve conduction velocity between baseline values and values at 1 hour, 2 hours, and 24 hours post-exercise. A trend was also found between the exercise-induced change in IL-6 and the change in NCV from baseline to 1 hour post-exercise (r=0.59, p=0.058, Figure 7) such that, the greater the increase in IL-6, the greater the increase in NCV. A positive correlation was found between the exercise-induced change in IL-6 concentrations and the change in NCV from
baseline to 2 hours post-exercise ($r=0.61$, $p=0.048$, Figure 8). Relative values were not analyzed due to multiple baseline testing IL-6 concentrations equaling 0 pg/ml.

5.5.2 Interleukin-10 & Nerve Conduction Velocity

Pearson’s $r$ correlations were performed to determine if any correlations existed between both the absolute and relative change in interleukin-10 concentrations and the change in nerve conduction velocity from baseline to 1 hour, 2 hours, and 24 hours post-exercise. No significant correlations were found between the change in IL-10 concentrations and the change in NCV at any time point, when examined as either the absolute or relative values.

5.5.3 Interleukin-1 Receptor Antagonist & Nerve Conduction Velocity

Pearson’s $r$ correlations were performed to determine if any correlations existed between both the absolute and relative change in IL-1ra concentrations and the change in nerve conduction velocity at 1 hour, 2 hours, and 24 hours post-exercise. A significant negative correlation was found between the change in exercised induced change in IL-1ra concentrations and the change in NCV from baseline to 24 hours post-exercise when examined under both absolute ($r=-0.65$, $p=0.021$, Figure 20) and relative ($r=-0.60$, $p=0.041$, Figure 21) values. Absolute values also yielded trends between the exercise-induced change IL-1ra concentrations and the change in NCV at 1 hour post-exercise ($r=-0.55$, $p=0.062$, Figure 16) and 2 hours post-exercise ($r=-0.48$, $p=0.11$, Figure 18) such that, the greater the increase in IL-1ra, the greater the decrease in NCV. Relative values also yielded a trend between IL-1ra and NCV at 1 hour post-exercise ($r=-0.49$, $p=0.109$, Figure 17) such that, the greater the increase in IL-1ra, the greater the decrease in NCV.
5.5.4 Tumour Necrosis Factor Alpha & Nerve Conduction Velocity

Pearson’s r correlations were performed to determine if any correlations existed between both the absolute and relative exercise-induced changes in TNF-α concentrations and the change in nerve conduction velocity at 1 hour, 2 hours, and 24 hours post-exercise. No significant correlations were found between the exercise-induced change in TNF-α concentrations and the change in NCV at any time points, when examined under either absolute or relative values.

5.5.5 Cytokines & Functional Measures

Pearson’s r correlations were performed to determine if any correlations existed between both the absolute and relative exercise-induced changes in each of the cytokines of interest and each of the functional measures investigated (strength, endurance and dexterity of the hand). A trend was found between the absolute exercise-induced change in IL-1ra concentrations and the change in handgrip endurance from baseline to 1 hour post-exercise (r=0.581, p=0.061, Figure 28). When examined with respect to the relative exercise-induced change a significant positive correlation was found between IL-1ra concentrations and the change in handgrip endurance (r=0.643, p=0.033, Figure 29) such that, the greater the increase in IL-1ra, the greater the increase in endurance scores.
VI. Discussion

6.1.0 Major Findings

The current study was the first to examine the potential effects of cytokines on peripheral nerve function in humans. The main finding is that two of the cytokines of interest were shown to be significantly correlated with alterations in nerve conduction. The positive correlation found between IL-6 and nerve conduction velocity approached significance 1 hour post-exercise ($r=0.586$, $p=0.058$) and achieved significance at 2 hours post-exercise ($r=0.606$, $p=0.048$). This positive correlation signifies that IL-6 may actually act to enhance nerve function as opposed to producing the negative channelopathic effects originally hypothesized. The negative correlation found between IL-1ra and nerve conduction velocity showed a trend 1 hour post-exercise ($r=-0.553$, $p=0.062$) and achieved significance 24 hours post-exercise ($r=-0.652$, $p=0.021$). This negative correlation signifies that IL-1ra may act to slow nerve function through potential channelopathic effects previously hypothesized. Interestingly, IL-1ra was also found to be positively correlated with endurance scores at 1 hour post-exercise ($r=0.643$, $p=0.033$), signifying that elevations in IL-1ra may improve muscular endurance despite its negative correlation with NCV. The cytokines IL-10 and TNF-α were significantly elevated following the exercise intervention but were not correlated with any change in NCV.

The potential conduction enhancing properties of IL-6 are novel findings and therefore possible mechanisms and rationale for this effect are not available in previous literature. However, based on the known response of IL-6 during exercise, it is possible to speculate a potential new role in the area of energy regulation. Skeletal muscle has been shown to act as an endocrine organ and produce IL-6 at times of an energy crisis when muscle glycogen stores become depleted. The secreted IL-6 then acts in a paracrine
fashion, stimulating the liver to increase hepatic glucose production and release it into the bloodstream, thereby supplying the skeletal muscle with an alternative fuel source (Febbraio et al., 2004). Our initial hypothesis that IL-6 would have a detrimental effect on NCV seemed fitting as this would imply that IL-6 regulates energy not only by controlling for energy production and release, but also by regulating energy usage. A decline in nerve conduction would reduce the physical abilities of an individual and help to reduce the energy demand to better match that of the available supply. However, as IL-6 was found to be correlated with a more rapid NCV it may actually be acting in an opposing fashion; enhancing nerve conduction to match the newly available supply of hepatic glucose. Likewise, it could be acting in a compensatory fashion, enhancing nerve function to atone for lower muscle glycogen availability.

The mechanism by which IL-6 may increase NCV is also currently unknown. However, when comparing IL-6 to other compounds, with more established mechanisms for enhancing nerve function, several similarities are apparent. Caffeine is a chemical known to enhance alertness and wakefulness. One such proposed mechanism for this is caffeine’s ability to block adenosine receptors along the cell surface (Kalmar & Cafarelli, 1999). Adenosine is a natural by-product of metabolism and acts to reduce the firing rate of neurons. By blocking these receptors caffeine prevents these effects and helps to increase neuronal firing rate. In addition to an increase in firing rate, the blocking of adenosine receptors causes stimulated nerve cells to release the hormone epinephrine. This release of epinephrine then acts to increase heart rate and blood flow to skeletal muscle, as well as cause the liver to release glucose. IL-6 has been shown to cause the release of hepatic glucose as well as the vasodilation of skeletal muscle arterioles which
would thereby increase skeletal muscle blood flow (Minghini, Britt, & Hill, 1998). It would therefore seem that IL-6 can produce some of the same effects as caffeine, but the question remains as to whether it accomplishes these effects through similar mechanisms. Interestingly, in a study performed by Minghini et al., it was shown that IL-6 only caused vasodilation when performed *in vivo*. As tests performed *in vitro* had no vasodilatory effects, it can be determined that IL-6 had no direct effects on vascular smooth muscle or endothelial cells, but instead must have involved parenchymal or intravascular factors (Minghini, Britt, & Hill, 1998). It is possible to speculate then, that IL-6 could in fact be causing this vasodilation through its direct effect on neurons, causing the release of secondary agents such as epinephrine. Further research is however necessary to establish this potential mechanism.

The negative correlation found between NCV and IL-1ra is in line with our original hypothesis as well as a previous study by Davies et al. (2006), which examined the potential effects of cytokines on neural function. Both the current study and that performed by Davies et al. (2006), found decreases in NCV when the cytokine of interest was at heightened concentrations. The current study differed from the Davies et al. (2006) study in several important aspects: i) the effects of a receptor antagonist (IL-1ra) rather than a cytokine with both pro and anti-inflammatory properties (TNF-α) was examined ii) the effects on peripheral nerves rather than central nerves were examined iii) human subjects were used rather than animal tissue iv) testing was performed *in vivo*, rather than *ex vivo*. The mechanism behind the change in nerve conduction found in these two studies may be similar as both showed a significant negative correlation between cytokine concentrations and NCV that was reversible. In the study performed by Davies
et al. (2006), as well as the present study the reversal of the cytokine-induced effects implies that structural damage was not the cause of the reduction in NCV and that an alteration in ion channel kinetics as seen in channelopathy was more likely to be the causative factor.

Despite the significant negative correlation between IL-1ra and NCV, no such correlation was found between IL-1ra and peak force production, or dexterity. It would therefore seem that although the elevated concentrations of IL-1ra achieved in the current study may prove to be adequate to cause reductions in NCV, they are not sufficient to translate into losses in the aforementioned functional measures. A significant positive correlation was however found between IL-1ra and muscular endurance. Interestingly, this finding suggests an improvement in muscular endurance when IL-1ra levels are elevated, despite the fact that elevated levels of IL-1ra were also correlated with reduced nerve function. It may be possible that a reduction in the frequency of nerve impulses (as caused by IL-1ra) allowed for the muscular contraction to be sustained for a greater period of time.

6.2.0 Clinical / Physiological Significance

It is important to note that both IL-6 and IL-1ra were successfully elevated to levels that closely mimicked those observed following SCI. For example, a study by Davies et al. (2007), showed subjects with SCI typically had levels of IL-6 that were approximately 3 times higher than healthy able-bodied controls and IL-1ra levels which were approximately 2 times higher. The change in TNF-α however, was only elevated 3-fold, in comparison to an approximately 8-fold increase following SCI. This may explain the lack of significant correlations involving TNF-α. The change in IL-10 also differed as
the Davies et al. (2007), showed no elevation in IL-10 following SCI whereas our study achieved a 3-fold elevation.

Although IL-1ra was shown to be negatively correlated with NCV and positively correlated with endurance, the acute elevation in each cytokine achieved during the current study did not appear to have substantial detrimental effects to physical function. It is possible however, that had the current study utilized functional measures that were more sensitive to small changes in physical function, significant changes could have been observed. The fact that IL-6 and IL-1ra were each correlated with NCV would justify such a speculation as an alteration in NCV would be expected to have some level of influence on function. It also must be taken into consideration that the current study observed acute elevations in cytokines, whereas following SCI, acute spikes caused by secondary health complications would be tied to a chronic elevation that persist at all times. As it is not uncommon for individuals with SCI to suffer from frequent secondary health complications, they are often in a state of chronic inflammation (Hayes et al., 2002). This would make individuals with SCI more susceptible to frequent reductions in physical abilities if particular cytokines such as IL-1ra are in fact responsible for reductions in NCV. In addition, as the physical abilities of these individuals may already be significantly compromised as a direct effect from neuronal damage, even a small drop in strength, endurance, or dexterity could have considerable implications. It is therefore not justified to conclude that cytokines could not have an effect on physical function within this population and future studies may still be warranted to examine this potential.

In addition to clinical significance, the results have physiological significance due to the potential neuromodulatory effects of IL-6. The fact that IL-6 was positively
correlated with NCV may indicate a greater role in energy regulation than originally believed. In addition to its role in stimulating hepatic glucose production and release, IL-6 may increase NCV to enhance physical functioning during bouts of exercise. This would provide important implications for IL-6 not only in special populations with chronically elevated cytokine concentrations, but also in healthy, able-bodied individuals. The negative correlation found between IL-1ra and endurance may also indicate an exercise related role through its potential effects on nerve function. The positive correlation observed between IL-1ra and endurance may be explained by a decrease in neuronal firing rate, allowing for force to be maintained for an extended duration.

Measures of nerve conduction velocity also play an important role as a predictor of outcome in the ICU. For example, motor nerve conduction velocity has been shown to be a significant mortality risk predictor in hemodialysis patients (Stosovic et al., 2008). The fact that disruptions in NCV are associated with higher mortality risks may actually be an indicator infection if cytokines are to have detrimental effects to nerve function. High levels of infection would invoke highly elevated cytokine levels which could then lead to disruptions in nerve signaling.

6.3.0 Reliability of NCV

In order to determine test-retest reliability of the nerve conduction velocity measure, the coefficient of variation between the familiarization session and baseline testing was calculated. An intra-class correlation coefficient of 0.416 was found. However, due to the homogeneity of subject means, resulting in low between-subject variability, an examination of the SEM was necessary. Calculation of the SEM resulted in a value of 2.87m/s. This value was then divided by the grand mean to calculate a
coefficient of variation of 5.19%. This coefficient of variation indicates a desirably low variability of NCV between testing days when compared to similar studies involving examination of NCV. For example, a study by Lew et al. (2005), examining the NCV on the median nerve produced a range of CV’s from 6.1% to 13.4%.

6.4.0 Reliability and sensitivity of ELISA’s

In order to assess intra-assay precision between duplicate measures, the correlation of variation of each sample was determined. Upon calculation of the coefficient of variation it was found that IL-6 had a value of 11%, IL-10 produced a value of 21%, IL-1ra produced a value of 22%, and TNF-α produced a value of 29%.

The sensitivity of each assay varied between cytokine and was determined by the minimum detectable dose (MDD) of the cytokine within the sample. IL-6 had an MDD of 0.70 pg/ml and IL-10 had an MDD of 3.9 pg/ml. The MDD of both IL-1ra and TNF-α ranged in values with IL-1ra falling between 2.15-18.3 pg/ml with a mean MDD of 6.25 pg/ml, and TNF-α falling between 0.5-5.5 pg/ml with a mean of 1.6 pg/ml.

6.5.0 Future Directions

This study was the first of its kind to examine the potential acute channelopathic effects of cytokines on peripheral nerve function, in vivo, using human subjects. Future studies that investigate the effects of chronically elevated cytokine levels of nerve conduction and functional tasks are also warranted. Such studies would be beneficial as it is unknown exactly how long each cytokine would take to ultimately produce its effects on neural function. It may therefore be possible to miss the potential channelopathic effects of cytokines when examining them under acute conditions.
It will also be necessary to test a wide variety of cytokines as the current study showed that the potential neural effects between cytokines can be highly variable. The fact that IL-6 was positively correlated with NCV whereas IL-1ra was negatively correlated may signify that certain cytokines may be beneficial to nerve function while others are detrimental.

Similar tests performed on individuals with SCI who are also suffering with some form of secondary health complication may also produce valuable findings as it would allow for the examination of nerve function while under extremely heightened cytokine elevations. This may also produce evidence as to the degree to which particular cytokines may affect nerve function and whether or not there is a ceiling effect. For example, if IL-6 does in fact enhance nerve function as suggested by the current study, it may be beneficial to learn by how much this effect is amplified under further heightened IL-6 levels, at what levels IL-6 ceases to have benefits, and whether extreme elevations can actually have negative effects.

Lastly, future studies may want to examine methods of manipulating cytokine concentrations, or limiting their effects. If particular cytokines are found to have harmful effects concerning nerve function, methods of lowering chronic elevations, or blocking their negative effects could be examined. Likewise, if certain cytokines are found to improve nerve function, strategies to enhance their effects or levels while minimizing the effects or levels of detrimental cytokines would be ideal.

**6.6.0 Limitations**

Several limitations were apparent in the current study. First, although the tests regarding functional measures were designed to determine if any substantial changes in
physical abilities existed, it may have been more appropriate to use more sensitive measures. When applying the findings to a special population such as SCI, it is important not to disregard small changes in function as even minute changes in strength, endurance, and dexterity can have immense implications concerning the ability to perform activities of daily living and therefore influence independence and overall quality of life. The use of more sensitive measures, particularly concerning the dexterity measurements, may have more accurately depicted small changes in function.

Second, alterations in NCV may have been more apparent had a longer nerve been utilized during testing. The use of a longer nerve within the leg would have forced the applied stimulation to travel a greater distance along the channelopathic nerve and may have made decreases in NCV more evident. It would however still be necessary to test on an inactive limb in order to help control for temperature and local metabolite production, so a unique exercise intervention would be necessary.

Finally, performing only one pre and post blood draw may have led to less accurate quantification of cytokine levels. Due to the fact that cytokine levels naturally fluctuate throughout the day, a more accurate measure of basal cytokine concentrations may have been possible had multiple pre-exercise blood draws been performed and averaged. Pre-blood draws were consistently taken at the same time of day between all subjects to help to account for fluctuations, however due to potential differences in sleep patterns and diet between subjects, multiple measures may have been beneficial. Multiple post blood draws may have also allowed for more accurate quantification of elevated cytokine levels. As different cytokines reach peak levels at varying time points following
the cessation of exercise, the true peak elevations of certain cytokines may have been missed.

6.7.0 Conclusion

The current study was the first to examine the acute effects of systemic cytokines on peripheral nerve function in humans. The results provided evidence for a potential nerve conduction enhancing role of IL-6. This may suggest an even greater role for IL-6 in energy regulation during exercise than originally believed. The results also provided evidence that IL-1ra may produce negative effects on nerve function and reduce NCV. The findings of this study warrant further research, potentially examining special populations with chronically elevated cytokine concentrations such as those with SCI. The fact that such populations have persistent elevations in systemic cytokines on top of pre-existing neurological deficits makes spinal cord injured individuals more susceptible to the effects of channelopathy.
References


Cytokine concentrations Prior to and Following Exercise Bout

Figure 4: Serum cytokine concentrations prior to and following 1 hour of aerobic exercise. Cytokine values are expressed as means ± SD and significance was set at p≤0.05. * denotes significant change from pretest to posttest as determined by a student’s t-test.
Nerve Conduction Velocity

![Graph showing NCV (m/s) prior to and at three time points following aerobic exercise. NCV values are expressed as means ± SD and significance was set at p≤0.05. * denotes significant change between NCV values obtained at 2h post-exercise and those obtained at 24h post-exercise (p=0.017).](image)

**Figure 5:** NCV (m/s) prior to and at a three time points following 1 hour of aerobic exercise. NCV values are expressed as means ± SD and significance was set at p≤0.05. * denotes significant change between NCV values obtained at 2h post-exercise and those obtained at 24h post-exercise (p=0.017)
Figure 6: Peak force production (kg) as measured by grip strength. Peak force values are expressed as mean ± SD and significance was set at p≤0.05.
Figure 7: Endurance as assessed by means of 30 second grip strength test. Endurance values are expressed as mean ± SD and significance was set at $p \leq 0.05$.  

Fatigue Index
Figure 8: Dexterity as assessed by a ten point finger tapping sequence. Dexterity values are expressed as mean ± SD and significance was set at $p \leq 0.05$. 
Dexterity Scores (Purdue Pegboard Test)

![Graph showing dexterity scores over time](image)

**Figure 9:** Dexterity as assessed by 30 second peg placement test. Dexterity values are expressed as mean ± SD and significance was set at p≤0.05. * denotes a significant difference between means such that a significant difference was found between pre-exercise and 2h post scores (p=0.003) and 2h and 24h post scores (p=0.047).
IL-6 – NCV Correlation between ΔIL-6 and ΔNCV: Absolute Change

A.

B.

C.

Figure 10: Correlation between absolute change of IL-6 and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
IL-10 – NCV Correlation between ΔIL-10 and ΔNCV: Absolute Change

A.

B.

C.

Figure 11: Correlation between absolute change of IL-10 and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
IL-10 – NCV Correlation between ΔIL-10 and ΔNCV: Relative Change

A.

B.

C.

Figure 12: Correlation between relative change of IL-10 and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
IL-1ra – NCV Correlation between ΔIL-1ra and ΔNCV: Absolute Change

A.

![Graph A]

B.

![Graph B]

C.

![Graph C]

**Figure 13:** Correlation between absolute change of IL-1ra and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
IL-1ra – NCV Correlation between ΔIL-1ra and ΔNCV: Relative Change

A.

B.

C.

Figure 14: Correlation between relative change of IL-1ra and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
TNF-α – NCV Correlation between ΔTNF-α and ΔNCV: Absolute Change

**A.**

Figure 15: Correlation between absolute change of TNF-α and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
TNF-α – NCV Correlation between ΔTNF-α and ΔNCV: Relative Change

**A.**

**B.**

**C.**

**Figure 16:** Correlation between absolute change of TNF-α and NCV. A) Represents changes 1h post exercise. B) Represents changes 2h post exercise. C) Represents changes 24h post exercise. Values are expressed as mean ± SD and significance was set at p≤0.05.
IL-1ra – Endurance Correlation: 1 Hour Post-exercise (absolute change)

Figure 17: Correlation between absolute change of IL-1ra and endurance 1 hour after the cessation of exercise. Values are expressed as mean ± SD. Significance was set at p≤0.05.
IL-1ra – Endurance Correlation: 1 Hour Post-exercise (relative change)

Figure 18: Correlation between relative change of IL-1ra and endurance 1 hour after the cessation of exercise. Values are expressed as mean ± SD. Significance was set at p≤0.05.
Appendices
Appendix A: Raw Data
**Table 2:** Cytokine concentrations (pg/ml) prior to and following 1 hour of cycling at 65% $\text{VO}_{2\text{max}}$.

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**Table 3:** Nerve conduction velocity (m/s)

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Table 4: Hand grip force production (kg)

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Table 5: Fatigue Resistance (kg) (final 5 sec / initial 5 sec)

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Table 6: Purdue Pegboard (# of successful pin placements)

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Table 7: Kapandji Test (time in seconds to complete sequence)

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<td>3.08</td>
<td>2.85</td>
<td>3.04</td>
<td>2.66</td>
</tr>
<tr>
<td>11</td>
<td>2.77</td>
<td>2.53</td>
<td>2.3</td>
<td>2.49</td>
</tr>
<tr>
<td>12</td>
<td>3.01</td>
<td>2.84</td>
<td>2.84</td>
<td>2.74</td>
</tr>
<tr>
<td>MEAN</td>
<td>2.81</td>
<td>2.74</td>
<td>2.72</td>
<td>2.66</td>
</tr>
<tr>
<td>SD</td>
<td>0.37</td>
<td>0.33</td>
<td>0.38</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Appendix B: ANOVA Tables & Correlation Matrices
**Table 8:** Pearson’s r Correlation – IL-6 – NCV (absolute change)

<table>
<thead>
<tr>
<th>IL-6 Absolute</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.586</td>
<td>0.607</td>
<td>0.440</td>
</tr>
<tr>
<td>r²</td>
<td>0.344</td>
<td>0.368</td>
<td>0.193</td>
</tr>
<tr>
<td>p</td>
<td>0.058</td>
<td>0.048</td>
<td>0.176</td>
</tr>
</tbody>
</table>

**Table 9:** Pearson’s r Correlation – IL-10 – NCV (absolute change)

<table>
<thead>
<tr>
<th>IL-10 Absolute</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.339</td>
<td>-0.226</td>
<td>-0.257</td>
</tr>
<tr>
<td>r²</td>
<td>0.115</td>
<td>0.051</td>
<td>0.066</td>
</tr>
<tr>
<td>p</td>
<td>0.281</td>
<td>0.481</td>
<td>0.420</td>
</tr>
</tbody>
</table>

**Table 10:** Pearson’s r Correlation – IL-10 – NCV (relative change)

<table>
<thead>
<tr>
<th>IL-10 Relative</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.091</td>
<td>-0.185</td>
<td>-0.179</td>
</tr>
<tr>
<td>r²</td>
<td>0.008</td>
<td>0.034</td>
<td>0.032</td>
</tr>
<tr>
<td>p</td>
<td>0.779</td>
<td>0.566</td>
<td>0.577</td>
</tr>
</tbody>
</table>

**Table 11:** Pearson’s r Correlation – IL-1ra – NCV (absolute change)

<table>
<thead>
<tr>
<th>IL-1ra Absolute</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.553</td>
<td>-0.485</td>
<td>-0.652</td>
</tr>
<tr>
<td>r²</td>
<td>0.306</td>
<td>0.235</td>
<td>0.426</td>
</tr>
<tr>
<td>p</td>
<td>0.062</td>
<td>0.110</td>
<td>0.021</td>
</tr>
</tbody>
</table>

**Table 12:** Pearson’s r Correlation – IL-1ra – NCV (relative change)

<table>
<thead>
<tr>
<th>IL-1ra Relative</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.486</td>
<td>-0.422</td>
<td>-0.595</td>
</tr>
<tr>
<td>r²</td>
<td>0.236</td>
<td>0.178</td>
<td>0.354</td>
</tr>
<tr>
<td>p</td>
<td>0.109</td>
<td>0.172</td>
<td>0.041</td>
</tr>
</tbody>
</table>
**Table 13:** Pearson’s r Correlation – TNF-α – NCV (absolute change)

<table>
<thead>
<tr>
<th>TNF-alpha Absolute</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.166</td>
<td>0.062</td>
<td>-0.092</td>
</tr>
<tr>
<td>r2</td>
<td>0.028</td>
<td>0.004</td>
<td>0.009</td>
</tr>
<tr>
<td>p</td>
<td>0.606</td>
<td>0.848</td>
<td>0.776</td>
</tr>
</tbody>
</table>

**Table 14:** Pearson’s r Correlation – TNF-α – NCV (relative change)

<table>
<thead>
<tr>
<th>TNF-alpha Relative</th>
<th>NCV 1h</th>
<th>NCV 2h</th>
<th>NCV 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.074</td>
<td>-0.015</td>
<td>-0.413</td>
</tr>
<tr>
<td>r2</td>
<td>0.006</td>
<td>0.000</td>
<td>0.170</td>
</tr>
<tr>
<td>p</td>
<td>0.818</td>
<td>0.963</td>
<td>0.182</td>
</tr>
</tbody>
</table>

**Table 15:** Pearson’s r Correlation – IL-1ra – Endurance (absolute change)

<table>
<thead>
<tr>
<th>IL-1ra Absolute</th>
<th>ENDURANCE 1h</th>
<th>ENDURANCE 2h</th>
<th>ENDURANCE 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.581</td>
<td>0.000</td>
<td>0.064</td>
</tr>
<tr>
<td>r2</td>
<td>0.337</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>p</td>
<td>0.061</td>
<td>0.999</td>
<td>0.852</td>
</tr>
</tbody>
</table>

**Table 16:** Pearson’s r Correlation – IL-1ra – Endurance (relative change)

<table>
<thead>
<tr>
<th>IL-1ra Relative</th>
<th>ENDURANCE 1h</th>
<th>ENDURANCE 2h</th>
<th>ENDURANCE 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.643</td>
<td>-0.029</td>
<td>0.081</td>
</tr>
<tr>
<td>r2</td>
<td>0.414</td>
<td>0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>p</td>
<td>0.033</td>
<td>0.933</td>
<td>0.813</td>
</tr>
</tbody>
</table>